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VOLUME 37 APRIL 1959 NUMBER 2

# PHORONIDEA FROM THE PACIFIC COAST OF NORTH AMERICA[1]

JOAN RATTENBURY MARSDEN

## Abstract

Six phoronids from the west coast of North America, *Phoronis vancouverensis*, *Phoronis pallida*, *Phoronis psammophila*, *Phoronis ovalis*, *Phoronopsis harmeri*, and one that is possibly *Phoronis architecta*, are described. *Phoronopsis harmeri* is considered to be synonymous with *Phoronopsis viridis*. Taxonomic relationships within the Phoronidea are discussed. It is suggested that the phylum may most naturally be divided into four categories of wide geographic range. The characteristics of each of these categories are discussed.

## Introduction

The phylum Phoronidea consists of a small group of animals all morphologically much alike. Since within this group there are few structural features which vary significantly one might expect the number of species to be small. Because of this limited degree of variation it is clearly desirable that any newly discovered population be examined with considerable care before it is assigned to a new or already described species. Unfortunately this ideal has not always been met. The first adult phoronids were described by Strethill Wright (21) in 1856; since then others have been discovered in many widely distant parts of the world and at times described as new species without adequate study of the animal or the pertinent literature. In 1939 Cori (4) listed 16 species, omitting *Phoronopsis californica* Hilton (9). Since then *Phoronis pallida* Schneider has been described by Silen (15), raising the number of species to 18. Both Cori and Silen comment on the dubious validity of some of the species in this list. Recently an examination of the literature, in an attempt to identify several phoronids from North America, has confirmed the inadequacy of a number of specific descriptions and has served to emphasize many uncertainties in the taxonomy of this group. Valuable suggestions concerning possible major taxonomic categories in the Phoronidea were made by Silen (15) and these will be considered in some detail in a later section of this paper.

Over a period of several years phoronids from the Pacific coast of North America were collected by or sent to the author and these collections provide the material for the present study. The localities represented range from Los Angeles in California north to Departure Bay on Vancouver Island.

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The results of this study have made necessary a re-evaluation of several specific descriptions and relate naturally to a discussion of the taxonomic structure of the phylum as a whole.

Phoronid taxonomy has been based on a more or less diagnostic set of morphological features, which seem to vary to a greater extent than do other aspects of the anatomy. Study of these features requires examination of both external and internal anatomy, the latter by means of selected serial cross sections. The morphological characteristics most commonly used are listed and briefly discussed below.

1. The length of the body. Phoronids vary in size, and in particular in length, from those which are no more than a few millimeters long to others which reach a length of 25 cm or more.

2. The number of tentacles. This is an extremely variable feature even within a single species. Differences between species may be of a different order, however, and helpful if used with discretion.

3. The shape of the lophophore. The double row of tentacles characteristic of the phoronid is borne on a connective tissue base known as the lophophore. The lophophore is always basically crescentic but the ends of the crescent may or may not be turned medially and coiled. The shape of the lophophore seems to be a constant feature within one species.

4. The number and arrangement of the longitudinal muscle bundles in relation to the mesenteries which divide the coelomic cavity in the trunk region. This is a feature which can be very helpful if used with due regard for the degree of variation normally found within any one species.

5. The number and nature of nephridial funnels. The paired nephridia, situated on either side of the anal papilla, end proximally in one or two open funnels. The funnels tend to develop expanded, ciliated edges and the size and relative elaboration of the funnels and ducts varies somewhat between species.

6. The nature and number of longitudinal nerve fibres. Most phoronid species possess one or two giant nerve fibers, which extend down the body from nerve cells in the region of the lophophore. In the case of phoronids with two such giant axons the fiber is usually very narrow (1.5 to 5 $\mu$ in diameter) whereas in forms with a single giant fiber the diameter may be much greater (9 to 27 $\mu$) (16).

7. The nature of the gonad. Phoronids may be hermaphrodite, protandric, or dioecious. Protandry is difficult to determine without detailed study but the decision between the hermaphrodite and dioecious conditions is straightforward and requires only that collections be made at suitable times of the year. The difference is obviously a basic one and too many species have been described without deciding this point.

8. The relative extent to which longitudinal and circular muscles are developed in the body wall. This feature is not frequently subject to variation, but in at least one type of phoronid it is highly modified and in this case of obvious taxonomic significance.

9. The presence or absence of an epidermal fold or collar at the base of the tentacular crown. This feature has been used as a criterion for separating the two genera, *Phoronis* and *Phoronopsis*, although there now seem to be a number of other characteristics (possibly numbers 6, 7, and 8 in this list) of comparable taxonomic importance.

## Materials and Methods

The Phoronidea concerned in this study come from a variety of sources. The *Phoronis vancouverensis* from the type locality at Departure Bay was taken from two collections. One of these collections was made by W. K. Fisher in 1922 and stored at Hopkins Marine Station (Pacific Grove, California), the other by myself in 1945–46. The Berkeley Harbor phoronid was collected by Mr. H. I. P. Pilgrim in 1953 and sent to me by Dr. R. I. Smith of the University of California at Berkeley. The Monterey Harbour and Mussel Point phoronids were collected by myself in 1949 and 1950. The *Phoronis vancouverensis* from San Juan Island were collected and sent by Mr. Russel Zimmer and Dr. Robert Fernald of the University of Washington (Seattle). The *Phoronis* from Coronal del Mar was collected and sent by Mr. W. E. Pequegnat of Pomona College (California). The collections from Los Angeles and Long Beach, California, were made and sent by Mr. Donald Reish of the Allan Hancock Foundation at Los Angeles, and the collections from Point Richmond, California, were made by Mr. Meredith Jones of the University of California at Berkeley. This latter collection was in exceptionally good condition, covered many months of the year, and provided excellent material for taxonomic study. *Phoronis ovalis* was collected on, and in the vicinity of, San Juan Island by myself and Dr. R. Fernald. The *Phoronopsis* from the coast of Washington and British Columbia were collected by Dr. R. Fernald, myself, Mr. and Mrs. C. Berkeley of the Pacific Biological Station, Nanaimo, B.C., and Dr. Swan, now at the University of New Hampshire.

Animals from each collection were studied intact (if possible) under the dissecting microscope and then by means of serial sections cut at 10 $\mu$. Some of the earlier studies were made on sections stained with haematoxylin and eosin. Later many were stained with protargol in an attempt to follow the details of the nervous system. Much of the material was bulk-stained in alum cochineal before sectioning.

All those who so kindly sent material, Dr. Smith, Mr. Zimmer, Mr. Reish, Mr. Jones, Dr. Fernald, Mr. Pequegnat, Mr. and Mrs. Berkeley, and Dr. Swan, also provided detailed information concerning localities, types of habitat, and associated organisms. This material was very helpful—some of it is included in the following account—and I would like to take this opportunity to thank those who contributed in this manner.

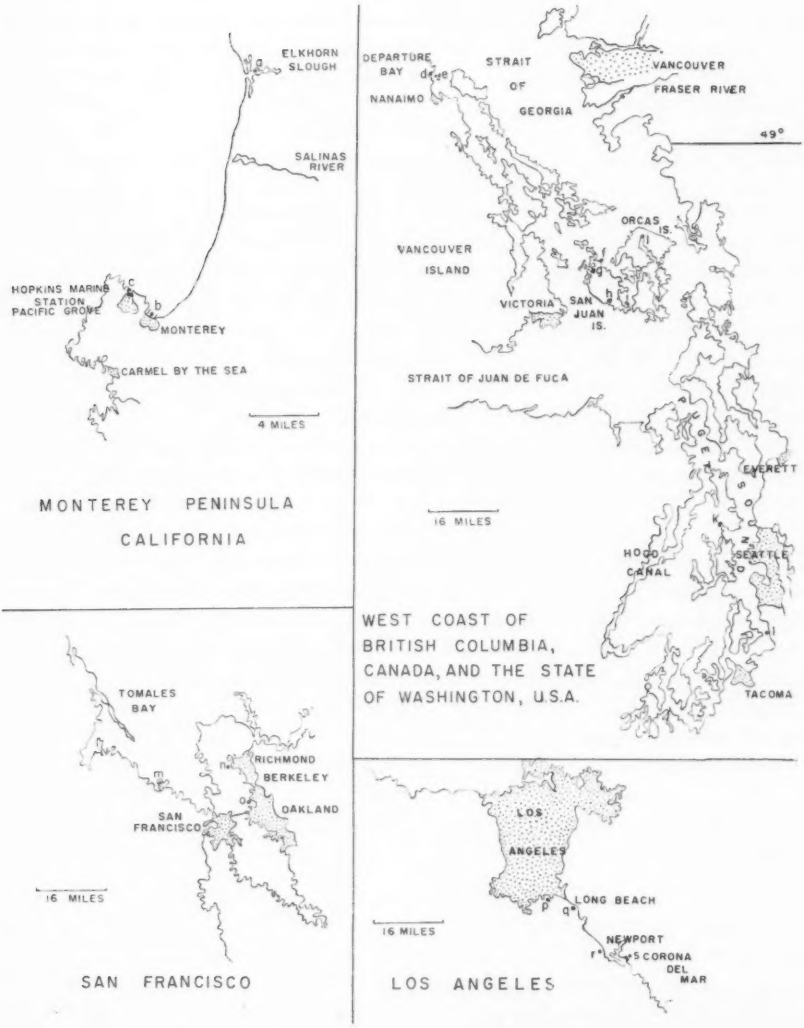


FIG. 1. The west coast of North America. Monterey Peninsula: (*a*) Elkhorn Slough, (*b*) Monterey Harbor, (*c*) Mussel Point; British Columbia and the State of Washington: (*d*) Departure Bay, (*e*) Strait of Georgia between Newcastle and Protection Islands, (*f*) Limestone Point (San Juan Island), (*g*) Garrison Bay (San Juan Island), (*h*) False Bay (San Juan Island), (*i*) Jaekyll's Lagoon (San Juan Island), (*j*) Crescent Beach (Orcas Island), (*k*) Miller Bay, (*l*) Des Moines Beach; San Francisco: (*m*) Bolinas Lagoon, (*n*) Berkeley Harbor, (*o*) Point Richmond; Los Angeles: (*p*) Los Angeles Harbor, (*q*) Long Beach, (*r*) Newport Bay, (*s*) Corona del Mar.

## The *Phoronis hippocrepia* Complex

Six populations of *Phoronis* have to be considered in this category. They come from Departure Bay (Vancouver Island), San Juan Island (Olympic Archipelago), Berkeley Harbor (California), Monterey Harbor (California), Mussel Point (Pacific Grove, California), and Corona del Mar (California) (Fig. 1). The Departure Bay population was collected at the type locality for *Phoronis vancouverensis*; the population from San Juan Island had been commonly known as *P. vancouverensis* and the other populations had never been identified as species.

All six populations exhibit the same type of growth form, a turf-like mass composed of many intertwined individuals with the tentacular crowns held up above the entangled tubes. In spite of this similarity different populations occur in very different ecological situations ranging from the surf-swept ledges at Mussel Point to the warm, quiet waters of Jaekyll's Lagoon on San Juan Island. A brief synopsis of the ecological conditions obtaining for the various populations concerned is given below.

Departure Bay population: On sandstone near low tide level in relatively quiet water; usually in dense clumps, occasionally in a thin encrusting layer.

San Juan Island: At Garrison Bay on limestone in quiet, rather muddy water; form dense clumps covered with silt. At Jaekyll's Lagoon on submerged logs in quiet, warm water; form loose clumps. At Limestone Point *in* limestone exposed to wave action and considerable tidal range.

Berkeley Harbor: On Yacht Harbor breakwater in rather quiet and polluted water; form dense, thick clumps.

Monterey Harbor: On old pilings at low tide level in moderately quiet water; form dense, thick clumps.

Mussel Point: In crevices and on ledges at low tide level in a region of rather heavy surf; form loose clumps.

Corona del Mar: On sand between reefs in 25 feet of water on the open coast; form dense clumps.

All six populations resemble one another and are, in fact, indistinguishable with respect to the size of the body, the number of tentacles, and the shape of the lophophore. The animals are usually 1–2 mm in diameter and get to be 35–40 mm long. The tentacle number varies as a rule between 100 and 200. The lophophore bearing the tentacles is in all cases horseshoe-shaped with the ends turned medially but not coiled (Fig. 2). No well-developed lophophore organ has been seen in any individual, although a modest thickening of glandular tissue near the bases of the inner tentacles has been seen in some cases.

The longitudinal muscles of the mid-body region are of the same type in all populations and consist of uniformly high central fibers and very low marginal fibers (Fig. 3). The total number and distribution of longitudinal

muscle fibers varies considerably. The range encompassed by measurements on 10–25 individuals from each population is as follows:

| | | | Totals |
|---|---|---|---|
| Departure Bay (20 specimens) | 13–26 | 19–24 | 43–68 |
| | 4–12 | 5–10 | |
| San Juan Island | | | |
| Limestone Point (15 specimens) | 15–23 | 20–28 | 51–63 |
| | 6–10 | 8–9 | |
| Jaekyll's Lagoon (10 specimens) | 21–25 | 23–25 | 66–67 |
| | 8–14 | 7–10 | |
| Garrison Bay (from M.A. thesis by Russel Zimmer) | 18–22 | 20–22 | |
| | 6–8 | 5–6 | |
| Berkeley Harbor (25 specimens) | 10–18 | 13–21 | 37–52 |
| | 5–8 | 5–8 | |
| Monterey Harbor (25 specimens) | 11–29 | 16–22 | 43–63 |
| | 3–9 | 4–7 | |
| Mussel Point (25 specimens) | 10–21 | 14–24 | 37–55 |
| | 2–11 | 3–9 | |
| Corona del Mar (20 specimens) | 12–21 | 13–20 | 44–50 |
| | 5–10 | 5–9 | |

The number and distribution of longitudinal muscle bundles are expressed according to the conventional formula of de Selys Longchamps (5) in which the four quadrants of the body wall, divided by longitudinal mesenteries, are represented as follows:

| Left lateral mesentery | Oral mesentery | | Right lateral mesentery |
|---|---|---|---|
| | Left oral coelom | Right oral coelom | |
| | Left anal coelom | Right anal coelom | |
| | Anal mesentery | | |

From this tabulation it is clear that (1) in any one population there is a wide range in total muscle number, (2) there is a great deal of overlap between the ranges for different populations, (3) the general pattern of distribution of muscle bundles along the four quadrants of the body wall is similar in all populations, and (4) the muscle formula for any individual from any population will not serve to identify that individual with the population from which it came.

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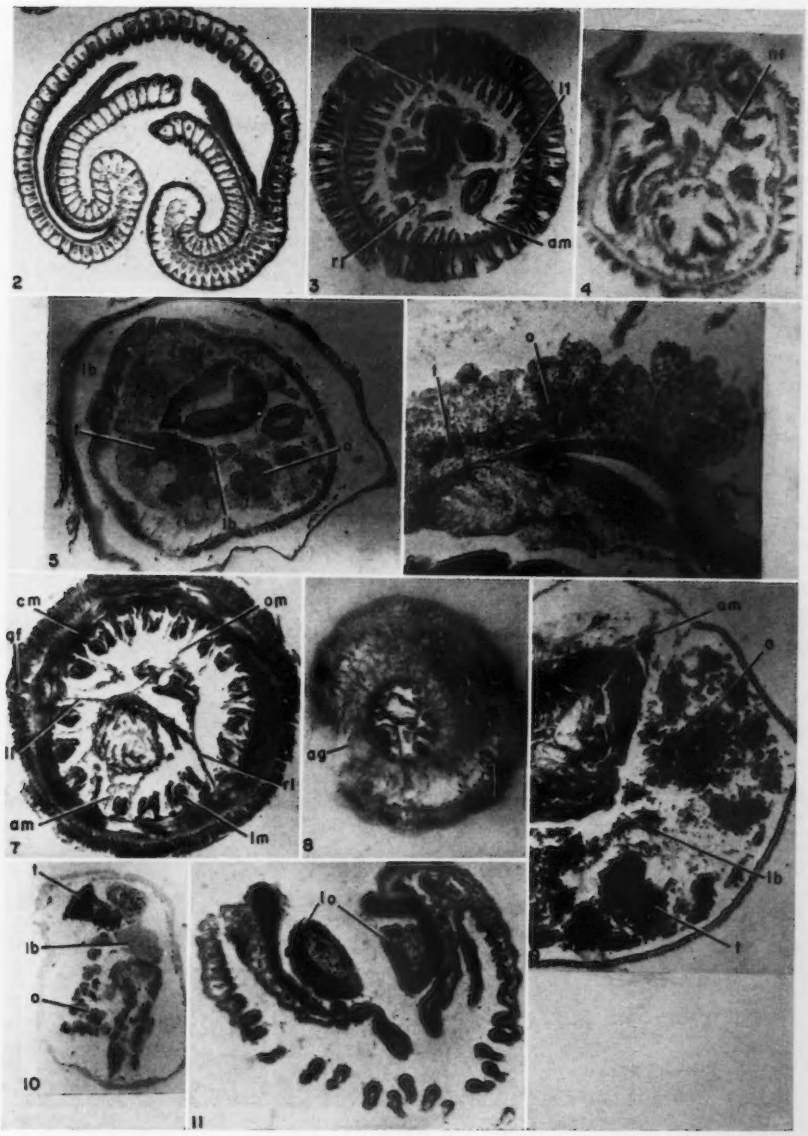
FIG. 2. *Phoronis vancouverensis* (San Juan Island). Cross section through the lophophore. FIG. 3. *Phoronis vancouverensis* (Mussel Point). Cross section through the mid-body region. FIG. 4. *Phoronis vancouverensis* (San Juan Island). Cross section through the nephridial funnels. FIG. 5. *Phoronis vancouverensis* (Monterey Harbor). Cross section through the reproductive funnels. FIG. 6. *Phoronis vancouverensis* (Departure Bay). Longitudinal section through the reproductive region. FIG. 7. *Phoronis pallida*. Cross section through the mid-body region. FIG. 8. *Phoronis pallida*. Cross section through the anal groove. FIG. 9. *Phoronis pallida* (Los Angeles). Cross section through the reproductive region. FIG. 10. *Phoronis pallida* (Point Richmond). Cross section through the reproductive region. FIG. 11. *Phoronis psammophila*. Cross section through the lophophore organ.

ABBREVIATIONS: *am*—anal mesentery, *ag*—anal groove, *cm*—circular muscle, *gf*—giant nerve fiber, *lb*—longitudinal blood vessel, *lm*—longitudinal muscles, *lo*—lophophore organ, *nf*—nephridial funnel, *o*—ovary, *om*—oral mesentery, *rl*—right lateral mesentery, *t*—testis.

PLATE I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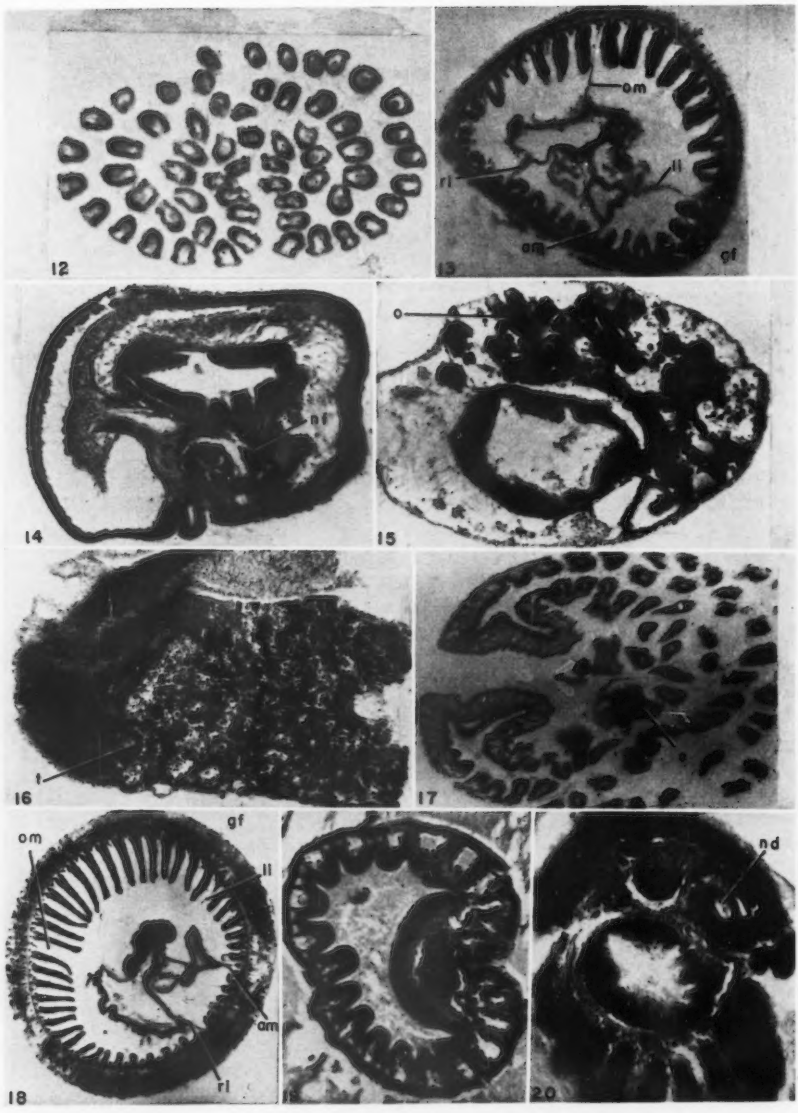


Marsden—Can. J. Zool.

With the exception of the San Juan Island group, individuals from all populations were sectioned and stained with protargol in an attempt to study the distribution of the giant nerve fibers. In all cases these fibers proved to be very small and difficult to follow with certainty. In fact they could be identified positively in only a few particularly favorable sections. Two longitudinal fibers could be seen in some sections; only one, the left, in others. Cori (4), de Selys Longchamps (6), and Silen (15) working with the somewhat similar European species, *P. hippocrepia*, provide contradictory evidence on the number of longitudinal nerve fibers, although Silen (15) concludes that *P. hippocrepia* probably has two giant fibers, both very small in diameter, the right being finer and shorter than the left. *P. vancouverensis* is very like *P. hippocrepia* in many respects and it seems likely that the condition of the giant fibers is very similar in the two species.

The morphological details of such nerve fibers do not provide suitable material for taxonomic study, since the difficulties involved in their examination must render comparative accounts unreliable. The bare fact that the longitudinal nerve fibers are small and difficult to follow in all of the six populations concerned serves to differentiate them from those phoronids in which the giant nerve fiber is a very large and easily seen structure. The distinction between the extremely fine giant fiber on one hand and the very obvious one on the other is very useful in phoronid taxonomy, but it seems that the finer details of the more delicate fibers belong in studies of detailed anatomy rather than taxonomy.

All individuals from all six populations were found to possess paired nephridia opening on either side of the anal papilla. The nephridium consists of a duct, doubled on itself once and ending in a pair of ciliated funnels, one opening into the oral, the other into the anal coelom (Fig. 4).

Cross sections through the genital region of individuals from all six populations indicate clearly that these animals are hermaphrodite, male and female tissue developing at the same time in one animal. In all cases the reproductive tissue lay in the left coelom, the male tissue on the oral side of the lateral blood vessel, the female on the anal side. The Monterey Harbor population was the only one in which the sectioned animals were in active breeding condition (Fig. 5), but the hermaphrodite condition of the immature gonads found in each of the other populations was very clear (Fig. 6).

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FIG. 12. *Phoronis psammophila*. Cross section through the lophophore. FIG. 13. *Phoronis psammophila*. Cross section through the mid-body region. FIG. 14. *Phoronis psammophila*. Cross section through the nephridial funnels. FIG. 15. *Phoronis psammophila*. Cross section through the female gonad. FIG. 16. *Phoronis psammophila*. Cross section through the male gonad. FIG. 17. *Phoronis psammophila*. Longitudinal section through the tentacular crown. FIG. 18. *Phoronis architecta*? Cross section through the mid-body region. FIG. 19. *Phoronis ovalis* (shell). Cross section through the lophophore. FIG. 20. *Phoronis ovalis* (shell). Cross section through the nephridial duct.

ABBREVIATIONS: *am*—anal mesentery, *e*—embryo, *gf*—giant nerve fiber, *ll*—left lateral mesentery, *lm*—longitudinal muscles, *nd*—nephridial duct, *nf*—nephridial funnel, *o*—ovary, *om*—oral mesentery, *rl*—right lateral mesentery, *t*—testis.

In all six populations there occur individuals bearing embryos in paired masses in the tentacular crown. Some of the details of this process have been observed both by myself and Mr. Russel Zimmer. The ova pass into the cavity of the tentacular crown intermittently throughout the breeding season, accumulate there, held to one another and the tentacles by a sticky secretion, and eventually are released as swimming actinotroch larvae.

The foregoing survey of the morphological features of six phoronid populations indicates no apparent means of separating one group from another. Although there is a considerable amount of variation in one feature, the number of longitudinal muscle bands, this variation is expressed within each population as well as within the total aggregate, making it impossible to use this feature in identifying any individual with any one of the six populations. It is true that the total range of longitudinal muscle number does not occur in each population, as sampled here, and that the low extremes of the range are found only in the Berkeley Harbor and Mussel Point populations, the high extremes in those from Departure Bay and Jaekyll's Lagoon. The overlap between populations is so great, however, that even if the measurements on an individual were to fall within the extremes of the range it would be impossible on that basis to place that individual in any one of the six populations. It remains possible, of course, that statistical analyses of larger numbers of individuals from each population might reveal significant differences between them. Even if this should be the case, however, it would mean that large numbers of individuals would have to be studied before the colony concerned could be placed in any one category. This, of course, would be an extremely cumbersome taxonomic procedure and it seems that populations that can be separated only by such elaborate means are best considered as varieties of a single species.

The only other obvious variable found in a study of these six populations concerns the ecological habit and it seems, therefore, that the six groups should be referred to a single species of *Phoronis* which must be considered to include a number of morphological and ecological varieties.

The Departure Bay population comes from the type locality for *Phoronis vancouverensis* Pixel and when the material studied here is compared with Pixel's (13) original description the fit is good except for the question of sexuality. Pixel saw only ova in the animals she examined, and concluded, therefore, that *P. vancouverensis* must be either protandric or dioecious. It must be pointed out here, however, that Pixel's specimens were collected in September, immediately after the end of the breeding season when the gonads were at a very low ebb. Her paper figures a cross section showing a few very small ova lying on the anal side of the lateral vessel and no reproductive tissue on the oral side where male tissue would be expected in accordance with material collected from the type locality and described here. In the immature gonad the ova are far more prominent than the developing male cells and the latter, unless the observer is very familiar with the detailed appearance of the developmental cycle in the gonad, may easily be mistaken

for vasoperitoneal tissue. Material collected from the type locality and agreeing reasonably with Pixel's description in every other respect is definitely hermaphrodite and it seems reasonable, therefore, to assume that the hermaphrodite nature of Pixel's specimens was not evident because of the season in which they were collected, and that the specific description for *Phoronis vancouverensis* should be corrected to include the hermaphrodite gonad.

If the six populations described here are included in the species *P. vancouverensis* the morphological scope of this species is extended to some degree. Several features are unaffected; the size of the body, the shape of the lophophore, the nature of the longitudinal nerve fibers, and the nephridia stand exactly as in Pixel's account. The range of variation in the number of tentacles, originally estimated at about 70–100 must be expanded upward to about 200 and the number of longitudinal muscle bundles, originally stated simply as 59–61, with a formula $\frac{19\text{–}24 \mid 22\text{–}24}{4\text{–}7 \mid 7\text{–}13}$, must be complicated and viewed as a variable situation with a total number of bundles varying at least from 37–68. A muscle formula can be set only by indicating the limits of variation found in the present study, which may be expressed as follows: $\frac{10\text{–}29 \mid 13\text{–}25}{2\text{–}14 \mid 3\text{–}10}$. Finally, as discussed above, the species must be considered to be hermaphrodite rather than protandric or dioecious.

If this expanded concept of *P. vancouverensis* is compared with descriptions for other species of *Phoronis* it becomes clear that the related European species, *P. hippocrepia* Str. Wright, comes extremely close to falling into the range of variation encompassed by *P. vancouverensis*. In fact the only non-conforming aspect of the description for *P. hippocrepia*, as set forth most recently by Silen (15), concerns the number of longitudinal muscle bundles, a feature already granted wide variability in the case of *P. vancouverensis*. Silen (15) and earlier de Selys Longchamps (6) state that the total number of longitudinal muscle bundles in *P. hippocrepia* varies from 31 to 38 with a muscle formula of $\frac{9\text{–}12 \mid 10\text{–}13}{4\text{–}6 \mid 5\text{–}7}$. The formula could be incorporated into the expanded one for *P. vancouverensis* by extending slightly the lower limit of variation in the oral chambers and by changing the range in total number from 37–68 to 31–68. With the above modifications the descriptions for the two species can be made to overlap completely. The European species is known to be hermaphrodite and de Selys Lonchamps (6) figures (Pl. 3, Fig. 3) a cross section in which the female cells lie on the anal side of the lateral blood vessel and the male cells on the oral side—a situation similar to that found in the six populations studied here. A further point of similarity concerns the ecological variation. *P. hippocrepia* may occur in tangled clumps on rocks or pilings or in holes in limestone rock. The latter is probably a rock-boring type similar to the Limestone Point population from San Juan Island.

There is considerable evidence in the literature to suggest that *P. hippocrepia* together with related or synonymous species forms a species complex widely distributed on European coasts and perhaps beyond. Ikeda (10) suggested that both *P. ijimai* (Japan) and *P. australis* (Australia) were synonymous with *P. hippocrepia*, but produced very little evidence in support of his argument. De Selys Longchamps (6) has synonymized *P. kowalevsky* with *P. hippocrepia*, and Cori (4) considers *P. psammophila* to be another synonym. Silen (15) considers *P. gracilis* and *P. hippocrepia* to be the same and believes *P. psammophila* to be a distinct but closely related species.

It seems, therefore, that the morphological, taxonomic, and ecological relationships of the North American *P. vancouverensis* are strongly reminiscent of the European *P. hippocrepia*. Having had no opportunity to study the European forms myself, I am not inclined to carry the argument further at this time. I would, however, like to emphasize the great similarity between these two groups and to suggest that we may perhaps be concerned with a single highly variable species complex of very wide geographical distribution.

## *Phoronis pallida* Schneider

The phoronids collected at Point Richmond (Fig. 1) by Meredith Jones fit, with one interesting exception, the description for *Phoronis pallida* Schneider (15). Many complete individuals collected at various times of the year were available for study and consequently it was possible to obtain a very clear picture of the morphology of the animal. In addition to the Point Richmond animals two incomplete individuals of the same type were found in the Los Angeles collections sent by Mr. Reish. Although neither of the specimens from this collection were whole, the morphology of this species is so distinctive that its identity is easily deduced from fragments of a reasonable size.

The phoronids from Point Richmond agree with the description for *Phoronis pallida* with respect to the size and shape of the body and tube, the shape of the lophophore, the nature of the small, paired nephridial funnels, the very large giant nerve fiber on the left side of the body (Fig. 7), the longitudinal muscle formula $\frac{5 \mid 5}{4 \mid 4}$, the division of the body by three horizontal sphincters of circular muscle, the differentiation of the longitudinal muscle into six zones, and the hermaphrodite condition of the gonads. The usual number of tentacles for individuals from the Point Richmond collection was about 50, occasionally more. Silen (15) describes 70–140 tentacles in the case of *P. pallida* from Sweden, but considering the usual degree of variation in this feature the difference is probably not of importance. Another minor point of difference concerns a groove found on the anal surface of the body in the Point Richmond specimens (Fig. 8). The groove tends to be widest at sphincter one, where it merges with the circular depression caused by the sphincter and tapers to nothing before reaching sphincter two. The groove was first noticed in cross sections and was later identified in the intact animal.

The most striking difference between the Point Richmond and Swedish forms, however, concerns the gonad. Both forms are simultaneous hermaphrodites. In the Swedish form (15) the ovary is situated on the oral and the testis on the anal side of the lateral blood vessel, in contrast to the condition in other hermaphrodite phoronids such as *P. hippocrepia*. In the Point Richmond form the position of the gonads is the reverse of the Swedish type: the testis is oral and the ovary anal with respect to the lateral blood vessel (Fig. 10). This condition is best seen in individuals collected somewhat prior to the peak of sexual maturity, since in fully ripe specimens the gonads occupy all available space and tend to become somewhat intertwined. On the other hand, one of the two fragments of *P. pallida* from Los Angeles includes the hermaphrodite gonad and in this case the ovary is oral in position and the testis is anal (Fig. 9). Of the two Los Angeles fragments, that one containing the gonad consists of body zones 4, 5, and 6 and the other of zones 2, 3, and 4 (15). These regions or zones are easily identified by reference to Silen's (15) description, and, in the aggregate, only one zone of the body, the most distal, is missing in the Los Angeles collection.

It would be desirable, of course, to be able to study a series of complete individuals from the Los Angeles area before drawing definite conclusions concerning the nature of the gonad in North American forms of *P. pallida*. On the basis of information available now, however, it looks as though the position of the ovary and testis relative to the lateral blood vessel may vary between different populations of this species. Certainly the Point Richmond type differs consistently from the Swedish forms, although there is apparently no other morphological difference of any importance. At this time, therefore, it seems best to consider the Point Richmond phoronid as a variety of *Phoronis pallida* Schneider, differing from the Swedish and Los Angeles forms with respect to the relative positions of the parts of the bisexual gonad.

### *Phoronis psammophila* Cori

From the Los Angeles and Long Beach collections sent by Mr. Donald Reish 48 complete or partial individuals were studied by means of serial cross sections. The examinations revealed four different types of phoronid. One of these, a variety of *P. pallida*, occurred rarely (two incomplete specimens) and has been discussed above. A second type, probably best considered as a variety of the European *Phoronis psammophila* Cori (3), was the most abundant of the four, 32 of the 48 individuals studied belonging to this type. The two remaining types found in the Los Angeles and Long Beach collections will be discussed in later sections of this paper.

Animals of the *P. psammophila* type were found in more or less straight tubes of fine, cemented sand grains. The tubes were about 2 mm in diameter and as most of those in the collection were obviously incomplete the probable normal length of the tube cannot be estimated. They occur in a muddy bottom and are not attached to one another in any way. Specimens

of this type were found in all three localities sampled by Mr. Reish, namely Los Angeles Harbor, Long Beach, and Newport Bay. They occur in fine grey clay at depths of 25 to 35 feet.

The largest complete individual found was 40 mm long, the muscular region of the body being very narrow, about 0.5 mm wide, and the proximal region somewhat thicker, about 1 mm in diameter. The muscular region of the body widens at the base of the tentacular crown, although there is no collar or epidermal fold in this region.

The usual number of tentacles was about 60–70. Most of the individuals examined possessed a lophophore organ (Fig. 11) in the form of a pair of high folds of tissue located medial to and including the bases of the inner row of tentacles. On the oral side of the body the lophophore organ takes the form of a thin membrane, which thickens anally to form a pair of conspicuous glandular folds. The lophophore itself is in the form of a horseshoe with slightly coiled ends (Fig. 12). The anal papilla, including anus and nephridial openings, tended to be prominent and high.

The number and type of longitudinal muscles is quite consistent within the group of animals studied and throughout the muscular body region of any one individual. The longitudinal muscle unit consists of a bundle of tall central fibers flanked by small lateral bundles of low fibers (Fig. 13). The longitudinal muscle formula varies as follows: $\frac{9 \mid 9}{4\text{–}5 \mid 4\text{–}5}$, the most frequent combination being $\frac{9 \mid 9}{4 \mid 5}$. There is a large, conspicuous and often folded giant nerve fiber on the left side of the body external to the attachment of the left lateral mesentery. This mesentery is usually complete throughout the muscular region of the body.

Each nephridium ends in a double funnel with modestly developed margins opening into both oral and anal coeloms (Fig. 14), the anal lip of the funnel being very small. Both male and female individuals have been found with well-developed gonads and there is no evidence suggesting either a protandric or a hermaphrodite condition. The animals certainly appear to be dioecious. The gonads develop, as in other phoronids, along the blood capillaries in the region of the ampulla and in both sexes the reproductive tissue lies on both oral and anal sides of the lateral blood vessel (Figs. 15 and 16). There appear to be no capillaries in the muscular region of the body. In three individuals embryos were found among the tentacles of the adult female (Fig. 17). In these cases the number of embryos was always small. In the case illustrated there were 10 embryos present, all blastulae and all slightly compressed. All three individuals concerned were ripe females with the distal end of the body packed with fully formed ova. A similar condition, small numbers of embryos at the same stage of development carried loosely in the tentacular crown of the female parent, has been described by de Selys Longchamps (6) for *Phoronis psammophila*. The functional significance of this condition remains obscure, however, and deserves a detailed study based

on living material. How long are the embryos kept in the tentacular crown? At what stage are they released? If a few ova are shed at one time and kept for a while among the tentacles of the parent is this performance repeated many times during the breeding season of a single female containing probably several hundred ova? Does the lophophore organ play a role in maintaining the ova in the tentacular crown, and how is fertilization achieved? De Selys Longchamps (6) reports that ova removed directly from the reproductive region by cutting the body wall will develop normally (he does not say how far), but otherwise the reproductive habits of *P. psammophila* remain unstudied and worthy of attention.

The description above is very like that for the European species *Phoronis psammophila* Cori. There are, in fact, only two points of difference apparent. The first concerns the question of the brood pouches described by de Selys Longchamps (6). According to that account the brood pouch, a thin membrane uniting the bases of the tentacles of the inner row, was characteristic of individuals bearing young in the tentacular crown. The lophophore organ, a glandular and more conspicuous structure, was found in some but not all such individuals. In the Los Angeles specimens a structure called here a lophophore organ was seen in all three animals bearing young, and in most but not all other individuals examined. In all cases this organ appeared to take the form of a thin membrane of closely packed columnar cells uniting the bases of the inner row of tentacles and expanding on either side of the anal papilla into a thickened fold of glandular tissue. In the Los Angeles specimens, therefore, the lophophore organ appears to be much the same in individuals bearing young and those that do not, and in all cases this organ appears to combine the essential feature of both the brood pouch and lophophore organ described by de Selys Longchamps for *P. psammophila.*

The second difference concerns the question of protandry. De Selys Longchamps (6) reports that with the exception of one individual all the specimens of *P. psammophila* studied by him (and he does not say how many) showed one gonad, either ovary or testis, occupying the entire reproductive region of the body. In the one exceptional case a group of young oöcytes were seen surrounded by male tissue. Because of this one individual de Selys Longchamps suggested that *P. psammophila* might be protandric, and this one animal in the process of change from the male to the female state. De Selys Longchamps did not, however, feel that protandry was by any means satisfactorily established for *P. psammophila* and continued to refer to this species as either protandric or dioecious. Obviously, here is another aspect of the natural history of this species that merits investigation. A year-long study based on frequent collections from one locality should serve to clarify the question of the nature of the gonad. Such a project has not yet been carried out and instead there has been a pronounced tendency among authors dealing with the Phoronidea to consider protandry to be a far more likely condition than dioecism, not only in *P. psammophila* but in most species which have not been shown to be simultaneous hermaphrodites.

In the case of the Los Angeles population the collections do not adequately cover a sufficiently long period to establish the nature of the annual changes in the gonad. Such evidence as there is indicates a dioecious condition during the breeding season without any evidence of protandry. Individuals with ripe gonads (male and female) were found in collections made in November and January, although some of the animals in the January collections showed only vasoperitoneal tissue in the reproductive region and appeared to be spawned out. In the June collections only females were found. Available information suggests, therefore, a fall and winter breeding season, but much more evidence is needed to complete the story.

This phoronid from California is then very like the European *Phoronis psammophila* and is probably best considered as a variety of this species. Lack of information, particularly concerning the reproductive cycle of both European and American forms, makes it impossible to examine both groups in an exhaustive manner. On the basis of the facts available at present, however, it seems best to consider the two forms as belonging to the same species, *Phoronis psammophila* Cori.

### *Phoronis architecta* Andrews

The third type of phoronid found in the Los Angeles collection was represented by only seven specimens. It was first separated from the more numerous specimens of *P. psammophila*, with which it was mixed, on the basis of the number of longitudinal muscle bundles. The number of bundles is considerably greater than it is in *P. psammophila* and the range established from the study of seven individuals can be expressed in the following formula, $\frac{15\text{–}16 \mid 15\text{–}17}{6\text{–}9 \mid 7\text{–}9}$, the most frequent combination being $\frac{15 \mid 15}{6 \mid 6}$ (Fig. 18). The longitudinal muscle bundles each consist of tall central fibers flanked by small masses of low lateral fibers. There is a single large giant fiber on the left side at the base of the left lateral mesentery. In one case, sections through the muscular region of the body were continuous with a series through the nephridium. Study of these sections indicated a nephridium with two rather small funnels. It seems very probable that some of the sections through the region of the ampulla must have been from this type of animal, but because none of the seven specimens were complete it was impossible to distinguish between sections through the reproductive region of *P. psammophila* and sections through the same body region of the third type of phoronid. If parts of the reproductive region of the third type of phoronid were represented in the sections studied it is likely that this third type is dioecious. It is impossible, however, to draw any conclusions on this matter at the present time.

The same relationship exists with regard to external anatomy. A study of the external features of the preserved animals in the collection failed to make any distinction between *P. psammophila* and the third type of phoronid. The only basis, therefore, for considering this type to be distinct from *P.*

*psammophila* is the number of longitudinal muscle bundles. This feature is, however, one of considerable taxonomic importance, and the number and arrangement of muscle bundles in the species *Psammophila* seem to be consistent within very narrow limits. With regard to the number of longitudinal muscle bundles the two types do not intergrade at all. The fact that no external difference was found is probably of no importance. The animals were preserved in acetone when received and therefore discolored and to some extent shrunken. There is not much external variation among phoronids anyhow and if the two types were approximately the same size and possessed roughly the same number of tentacles it might well be impossible to separate them in the preserved condition on the basis of external appearance.

It is interesting to note here that in an unpublished report by Russel Zimmer there is mention of a possibly similar phoronid found in the Tacoma Aquarium at Point Defiance (Washington) in the spring of 1954. The animals are reported to inhabit leathery tubes intertwined with the calcareous tubes of serpulid polychaetes. The muscle formula is stated as $\frac{16 \mid 16}{6 \mid 6}$. All animals available in the aquarium were collected by Mr. Zimmer at that time.

If the longitudinal muscle formula for this third type is compared with descriptions for other species of phoronid it is found to resemble most closely that for *Phoronis architecta* (1), a species from the coast of North Carolina with a muscle formula as follows: $\frac{13 \mid 12\text{–}16}{4\text{–}9 \mid 5\text{–}6}$. The two forms are also similar with regard to the presence of a single, large giant nerve fiber, the nature of the nephridial funnels, and the possibility of a dioecious gonad. Obviously there is not enough information available on the third type from California to identify it with any known species. It seems, however, that if and when more information becomes available this form should be compared with the description for *P. architecta*, with which it appears to have many similarities.

## *Phoronis ovalis* Str. Wright

*Phoronis ovalis* was first found in the Puget Sound region by Mr. Russel Zimmer, who saw small phoronids living in holes in shells of *Pododesmus* in the aquarium at Point Defiance Park in Tacoma, Washington. Later he found *P. ovalis* in a variety of shells (*Pododesmus*, *Crepipatella*, *Balanus nubilis*, *Crepidula*) dredged from Puget Sound and in the limestone cliffs at Limestone Point on San Juan Island.

During the summer of 1956 I was able to collect *P. ovalis* in Puget Sound myself. The animals were particularly common in dredged shells of *Pododesmus*, *Hinnites*, and *Balanus nubilis*. Sixteen individuals taken from shells were sectioned and studied, together with nine individuals taken from Limestone Point (collected in September 1956 by Dr. R. Fernald).

The two populations appear to belong to the same species. In both cases the animals ranged in length from 4–10 mm. The number of tentacles varied from 18–22 in the shell specimens, and from 17–19 in the limestone forms. In both cases the lophophore took the form characteristic of this species, an indented circle (Fig. 19). The nephridia in both populations were straight ducts opening close together on the anal papilla and diverging proximally to end each in a single ciliated funnel in the lateral body cavity (Fig. 20). In all cases an oesophageal valve was present near the level of the nephridial funnels (Fig. 23). The longitudinal muscles varied in number from 24–36 in the shell population, and from 28–36 in the limestone forms. In all cases the longitudinal muscles appeared to be divided into two equal groups by the oral and anal mesenteries (Fig. 23). Lateral mesenteries and giant nerve fibers were absent. The gonad, lying in the proximal region of the body, was in all cases immature. Russel Zimmer reports finding ova in the body cavity from August to November (1955) and one larva in March. I did not see any ova or larvae during the summer of 1956 and it seems likely that this animal reproduces sexually during the winter. In the summer of 1956 (July and August) many of the animals found in shells were actively reproducing by means of transverse fission. In the sectioned material a well-developed male gonad (not entirely mature, but extensive) was found in several specimens from the shell group (Fig. 21), and one individual from the limestone population showed small and decidedly immature ova (Fig. 22). In no case were both male and female gonads found in one animal. These results do not support Silen's (15) description of an hermaphrodite, simultaneously active gonad, although the apparent condition of the Puget Sound *P. ovalis* (male gonad in July, female in September) is reminiscent of the description for *P. ovalis* (12) from Brazil (testis in March, ovocytes in July).

The Puget Sound *P. ovalis* were examined for the "third longitudinal blood vessel" described by Marcus (12) from the Brazil material. No such vessel was found, although groups of blood corpuscles associated with the oral side of the descending limb of the digestive tract were occasionally found. These corpuscles appeared, however, to be in the irregularly filled blood sinus found in the wall of all parts of the digestive tract, and no consistent or distinct third longitudinal vessel was seen.

### *Phoronopsis harmeri* Pixel

In the course of study of the phoronids sent from Los Angeles seven specimens of a *Phoronopsis* were found which appeared to fit the description for *Phoronopsis harmeri* (14). At this time the description for *P. harmeri* was compared with that for *Phoronopsis viridis* Hilton (8) and the two were found to be essentially the same. Hilton (8) claimed that his species was distinct from any other, including *P. harmeri*, but did not specify the differences between the two species. A comparison of the two accounts reveals descriptions similar with respect to size of body and tube, number of tentacles, color of tentacles (pale green), shape of lophophore, presence of a single

giant fiber on the left side of the body, and number of longitudinal muscle bundles. Pixel (13) gives one longitudinal muscle formula and Hilton (8) gives two, one slightly smaller, the other slightly larger than Pixel's. Hilton gives no account of the details of the nephridial funnels or the nature of the gonad in *P. viridis*.

In the intertidal mud flats of central and southern California there occurs a large *Phoronopsis* bearing green tentacles and commonly known as *P. viridis* (originally described from Moro Bay, southern California). Earlier studies of my own (14) on this animal as found at Elkhorn Slough and Bolinas Lagoon (central California) reveal its similarity to Hilton's brief description and include accounts of the structure of the nephridia and the annual cycle of changes in the gonad. If this description of the nephridia and gonads (14) is added to Hilton's description of *P. viridis* there remains no clear ground for difference between the descriptions for *P. viridis* and *P. harmeri*. The nephridium, bearing two funnels of unequal size, is the same in both cases. *P. viridis* is definitely dioecious (14) and *P. harmeri* is described as being probably dioecious (Pixel saw only the female gonad). Specimens collected at Departure Bay (type locality for *P. harmeri*) by the author are clearly dioecious.

It seems, therefore, that the two species *P. viridis* and *P. harmeri* cannot be maintained as separate types; that the animal concerned must go under the earlier name *Phoronopsis harmeri* and that the name *viridis* must be dropped and declared synonymous with *harmeri*.

In addition to the specimens from California, studies were made on animals taken from several populations of *Phoronopsis* found on the coasts of the State of Washington and the Province of British Columbia. This group included 26 individuals from Miller Bay (Washington), 5 individuals from Des Moines Beach (Washington), 5 from Crescent Beach (Washington), 4 from False Bay (Washington), 5 from Departure Bay (British Columbia), and 3 taken in 1912 from the Strait of Georgia between Newcastle and Protection Islands and found in the collection of the Pacific Biological Station at Departure Bay, Vancouver Island. All the localities quoted above are shown in Fig. 1.

The six populations from Washington and British Columbia appear to belong to a single species. They are similar to one another and to the California *Phoronopsis* with respect to the presence of an epidermal collar (Fig. 24), the length and diameter of the body, the shape of the lophophore (Fig. 25), the size and position of the giant nerve fiber, the nature of the nephridium, and the longitudinal muscle bundles. All six populations were shown to be clearly dioecious, individuals being distinctly either male or female with the exception of the specimens from the collection in the Pacific Biological Station. None of the latter were both complete and sexually mature, and this collection yields, therefore, no evidence on the question of sexuality. Living animals were seen at Miller Bay, Des Moines Beach, Crescent Beach, and Departure Bay, and in all cases the tentacles were a pale green usually flecked with white.

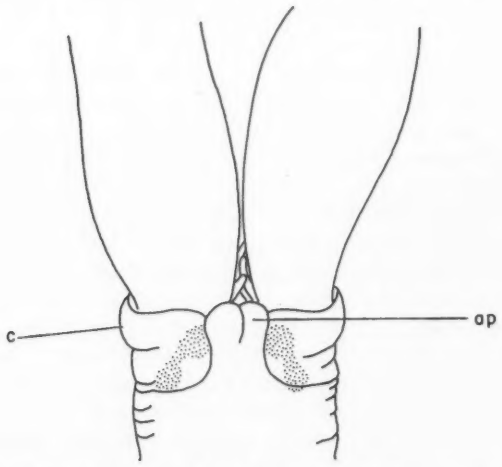


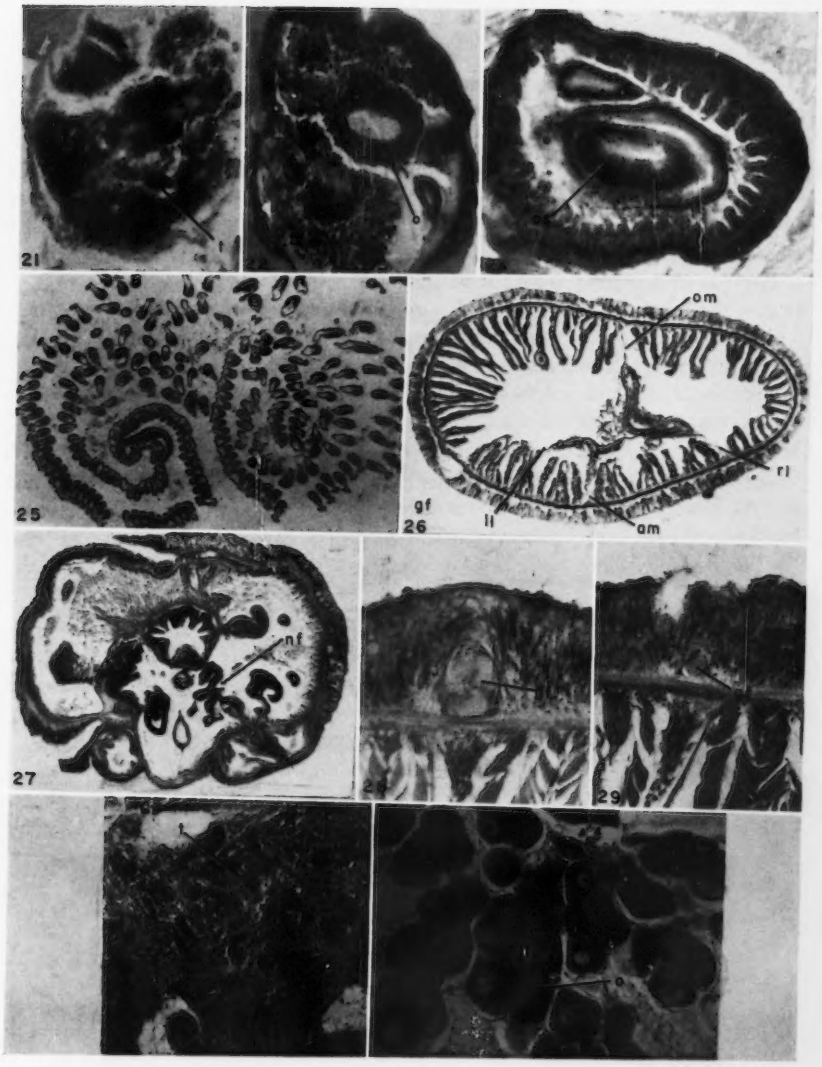

FIG. 24. *Phoronopsis harmeri.* The anal surface of the distal end of the body. ABBREVIATIONS: *ap*—anal papilla, *c*—collar.

It seems, therefore, that there is one relatively common species of *Phoronopsis* found along the Pacific coast of North America from southern California north to Vancouver Island. The proper name for this species is *Phoronopsis harmeri* Pixel and an expanded description based on a study of individuals from eight populations is given below. These populations include the six discussed above, as well as one from Elkhorn Slough (18 specimens), and the 7 individuals from Los Angeles.

*Phoronopsis harmeri* is generally found in a substrate of mixed sand and mud. The tubes are arranged more or less vertically and are usually about 4–6 cm long. The lower, proximal end of the tube is closed and usually abruptly tapered. The upper end is open and may project above the surface or lie under several inches of sand and mud. When the substrate consists of fine-grained sand and mud, the tubes tend to be straight, of uniform diameter, and are composed of delicate, neatly cemented grains. At some localities, such as Departure Bay or Crescent Beach, there is a considerable amount of gravel mixed with the sand and mud of the beach, and at such

FIG. 21. *Phoronis ovalis* (shell). Cross section through the male gonad. FIG. 22. *Phoronis ovalis* (limestone). Cross section through the female gonad. FIG. 23. *Phoronis ovalis* (shell). Cross section through the oesophagus. FIG. 25. *Phoronopsis harmeri* (Los Angeles). Cross section through the lophophore. FIG. 26. *Phoronopsis harmeri* (Los Angeles). Cross section through the mid-body region. FIG. 27. *Phoronopsis harmeri* (Elkhorn Slough). Cross section through the nephridia. FIG. 28. *Phoronopsis harmeri* (Elkhorn Slough). Left giant nerve fiber in cross section. FIG. 29. *Phoronopsis harmeri* (Elkhorn Slough). Right giant nerve fiber in cross section. FIG. 30. *Phoronopsis harmeri* (Elkhorn Slough). Cross section through male gonad. FIG. 31. *Phoronopsis harmeri* (Elkhorn Slough). Cross section through female gonad. ABBREVIATIONS: *am*—anal mesentery, *gf*— giant nerve fiber, *ll*—left lateral mesentery, *nf*—nephridial funnel, *om*—oral mesentery, *os*—oesophageal sphincter, *rl*—right lateral mesentery, *t*—testis.

PLATE III




Marsden—Can. J. Zool.

places the tube tends to be twisted and to incorporate into its wall pebbles considerably larger than the diameter of the lumen, resulting in a lumpy, irregular construction.

The tentacles range in number from about 100 to 300 and are about 2 mm long. There is a distinct collar at the base of the tentacular crown (Fig. 24), best developed on the anal surface. The ends of the lophophore are coiled (Fig. 25) and no lophophore organs were seen.

There is a very large giant nerve fiber on the left side of the body (Fig. 28) and in some cases a much finer and shorter fiber was found on the right side (Fig. 29). The right-hand fiber was found quite consistently in the Elkhorn Slough population and in a few individuals from the Crescent Beach and Miller Bay populations. The condition of the tissues at the time of staining and the stain used probably have considerable effect on the appearance of the fine right-hand fiber. It seems probable that the presence of the right-hand fiber is characteristic of *P. harmeri*, but a more detailed histological study of several populations would be necessary to settle this point.

Each of the paired nephridia bear two funnels, a relatively large one opening into the oral coelom and a smaller, higher one opening into the anal coelom (Fig. 27). Serial sections through single individuals indicate that the number of longitudinal muscle bundles varies within one individual, being greatest at the lower end of the muscular region, just above the ampulla. The number of longitudinal muscle bundles decreases toward the distal end of the body, as is indicated by the following three formulae based on counts from a single individual in the Los Angeles collection:

$$\text{Proximal} \rightarrow \text{distal} = \frac{37 \mid 35}{20 \mid 18} \rightarrow \frac{33 \mid 32}{20 \mid 18} \rightarrow \frac{29 \mid 30}{16 \mid 15}.$$

The third formula was consistently applicable to the longest part of the muscular region of the body (Fig. 26) and counts of muscle bundles for comparative purposes (in a series of different individuals) were always made in this region of the body. Composite muscle formulae made in this way for each of the eight populations are given below:

| | | |
|---|---|---|
| Los Angeles (7 individuals) | $\frac{20\text{–}46 \mid 23\text{–}40}{13\text{–}24 \mid 13\text{–}22}$ | = 79–126 |
| Elkhorn Slough (18 individuals) | $\frac{26\text{–}42 \mid 26\text{–}46}{14\text{–}23 \mid 15\text{–}22}$ | = 82–131 |
| Miller Bay (26 individuals) | $\frac{32\text{–}48 \mid 31\text{–}45}{19\text{–}25 \mid 15\text{–}22}$ | = 104–138 |
| Des Moines Beach (5 individuals) | $\frac{34\text{–}42 \mid 32\text{–}40}{19\text{–}22 \mid 16\text{–}20}$ | = 101–117 |
| Crescent Beach (5 individuals) | $\frac{28\text{–}40 \mid 32\text{–}40}{20\text{–}22 \mid 18\text{–}20}$ | = 101–122 |
| False Bay (4 individuals) | $\frac{34\text{–}43 \mid 36\text{–}46}{18\text{–}24 \mid 15\text{–}20}$ | = 107–128 |
| Departure Bay (5 individuals) | $\frac{37\text{–}46 \mid 34\text{–}55}{15\text{–}27 \mid 14\text{–}22}$ | = 102–135 |
| Pacific Biological Station (3 individuals) | $\frac{28\text{–}48 \mid 31\text{–}55}{16\text{–}27 \mid 14\text{–}22}$ | = 101–138 |

The specimens from California tend to be somewhat smaller than those from the northwest and this difference is reflected in a smaller average number of longitudinal muscle bundles in the former group. The extent of overlap between all groups is so great, however, and they are so similar in other respects that it seems best to place all populations within the single species *P. harmeri*.

All six populations have been seen to be dioecious (Figs. 30 and 31).

In addition to the six populations of *Phoronopsis* studied by means of selected cross sections other populations, probably belonging to the same species, are known either from personal collecting or from the reports of others. A *Phoronopsis* externally similar in every way to *P. harmeri* has been collected at Moro Bay, Bodega Bay, and Bolinas Lagoon, all in California (Fig. 1). In these cases the animals occur as they do at Elkhorn Slough, in very extensive beds in the lower intertidal zone. North of California a single specimen of *Phoronopsis* was taken at Minnesota Reef (San Juan Island) and another near Roche Harbor (San Juan Island). A similar animal has been collected in larger numbers from Whidby Island, Washington, by Dr. R. Fernald. In addition Mr. Russel Zimmer reports finding a *Phoronopsis* at Cute Island (San Juan Island) and a larger population at Bainbridge Island, Washington. The latter population was found in 35 feet of water and the diver, Mr. Paul Sund, reports an extensive bed of these animals in that area.

Mr. Russel Zimmer in an unpublished report gives the following muscle formulae for individuals from Bainbridge Island and Cute Island respectively: $\frac{47 \mid 43}{22 \mid 19}$ and $\frac{30 \mid 30}{16 \mid 16}$. In the case of the Bainbridge Island form Mr. Zimmer reports a large left lateral nerve fiber and two tentacle counts lying within the 200–300 range.

The available evidence, therefore, suggests that *P. harmeri* is the common *Phoronopsis* found in intertidal and shallow subtidal waters along the Pacific coast of North America.

## Discussion

Six species of phoronid from the west coast of North America have been considered in the preceding section of this paper. If the geographic ranges of each of these is plotted it will appear that four of the six have an essentially cosmopolitan distribution.

Each of the cosmopolitan species, *P. hippocrepia*, *P. psammophila*, *P. pallida*, and *P. ovalis*, has been shown to include some degree of morphological variation. It looks, therefore, as though we may be concerned here with four basically different taxonomic units each of which exhibits extensive geographic dispersal and some anatomical variation.

In a paper dealing with phoronids found in the Gullmar Fiord area (Sweden), Silen (15) is also concerned with four species; they are *P. pallida*, *P. ovalis*, *P. hippocrepia*, and *P. mulleri*. Silen points out that all four are well-defined species, each as distinctive in its own right as is the genus *Phoronopsis* set up

by Gilchrist (7) for *Phoronopsis albomaculata* from South Africa, and suggests that the phylum may possibly be divided most reasonably into five categories represented by the four species of *Phoronis* found in the Gullmar Fiord plus the genus *Phoronopsis*. Silen also suggests that other described species may well be found to fit into one or other of these categories, when studied carefully.

The fact that on the west coast of North America we have the genus *Phoronopsis* as well as three of the four species of *Phoronis* found in the Gullmar Fiord lends support to Silen's ideas. The information available from North America at the present time suggests, in my opinion, four major categories largely but not entirely similar to those suggested by Silen.

*Phoronis ovalis*, as found in Europe, Brazil, New Zealand (19), and North America constitutes one of these categories. This group seems to be adequately defined by special features such as the absence of giant fibers and lateral mesenteries, the virtual suppression of the larval stage in the course of sexual reproduction, the production of relatively small numbers of relatively large ova (17), and the characteristic seasonal alternation of sexual and asexual modes of propagation (17 and 18). The nature of the gonad, hermaphrodite, dioecious, or protandric, may possibly vary between European and American forms, but information on the latter populations is inadequate at present. The habit of burrowing into shells or limestone is a characteristic shared to some extent by the *hippocrepia* group discussed below. The *ovalis* group is usually sublittoral in distribution, although the population from Limestone Point on San Juan Island forms an exception to this rule.

*Phoronis pallida*, found both in Europe and North America, forms the second category. This group is rendered distinctive by the specialization of the body-wall muscles, resulting in six different body zones, and by the nature of the tube, which is open proximally. Other less-distinctive characteristics of this group are the large giant fiber, the hermaphrodite nature of the gonad, the small size of the ova, the relatively long larval life (17), and the fact that the animals occur as separate, unattached individuals placed vertically in a mixture of sand and mud. Silen (15) states that *P. pallida* occurs in a somewhat firmer bottom than does *P. mulleri* in the same region and there is perhaps an ecological distinction here, related possibly to the open tube of *P. pallida*.

The remaining species of phoronids appear to me to fall into two fairly well-defined categories. The first of these has been referred to in this paper as the *P. hippocrepia* complex. Already synonymized with *P. hippocrepia* are *P. gracilis* (15), and possibly *P. australis* and *P. ijimai* (10). *P. vancouverensis* is obviously very similar and *P. capensis* (7), a limestone-dwelling form, seems from its description to be very like *hippocrepia*, although there is no account of the gonad of *capensis*—a serious deficiency. The *Phoronis hippocrepia* group is characterized by giant nerve fiber(s) (one or two) of small diameter (4–5 $\mu$), a simultaneously hermaphrodite gonad (ovary on the anal side of the lateral blood vessel, testis on the oral side), a yolky egg intermediate in size and abundance (17), the habit of brooding the young in paired masses in the

tentacular crown (each mass including up to about 50 embryos ranging from newly shed, uncleaved ova to actinotroch larvae ready for release to the plankton), a relatively short larval life (17), and the characteristic growth form, a tangled mass of leathery tubes adhering to one another and forming either a felt-like mat attached to a firm surface or a series of winding, intimately related tubes occupying channels in limestone rock. This group has been found both in littoral and sublittoral zones. Ontogenetically the growth habit may be the result of a tendency for the larva to settle on a hard substrate (usually rock or wood). This relationship was suggested by Brooks and Cowles (2) and is further supported by studies on the larva by Silen (17).

The fourth and final category suggested here includes the genus *Phoronopsis* as well as *Phoronis architecta*, *Phoronis mulleri*, *Phoronis psammophila*, and possibly others. After a careful study of the description of *P. psammophila* (3, 4, and 6) I cannot agree with Silen's (15) conclusion that this species is most closely related to *P. hippocrepia*, but prefer to include it in this fourth group. This is probably the least sharply defined of the four categories; it appears at present to include the greatest variety of forms and it may well be that the accumulation of more information in the future may justify its subdivision. The forms referred to this group do not possess the special features of either the *ovalis* or *pallida* categories, and they differ from the *hippocrepia* group in that the giant nerve fiber(s) (one or two) are of large diameter (9–27 $\mu$) and the animals occur as separate individuals placed more or less vertically in sand or mud. The growth habit is probably related to the tendency of the larva to settle on a non-solid substrate. With the exception of *P. mulleri*, the species referred to this category have not been found to be simultaneous hermaphrodites. The status of *P. mulleri* in this respect is unsatisfactory at present. De Selys Lonchamps (5 and 6) claims to have seen only the female gonad. He found neither male nor hermaphrodite individuals, but does not quote the number of animals examined. Silen (15), in 1952, speaks of *P. mulleri* as a species in which "only one type of gonades has been found in one and the same specimen" and concludes that the animal may possibly be dioecious. He mentions no histological studies of his own on this species and gives no figures of the gonad. In a later paper, Silen (17) claims that *P. mulleri* is hermaphrodite (describing it and *P. pallida* in the same terms and the same sentence), but uses only his 1952 (15) paper as documentation. This contradiction in statement and the lack of documented evidence of hermaphroditism in *P. mulleri* results in a need for clarification concerning the nature of the gonad in this species. The nature of the gonad is likewise unknown in three species of *Phoronopsis*: *P. albomaculata* (7), *P. californica* (9), and *P. striata* (8). *Phoronopsis harmeri* (*viridis*) is clearly dioecious (14) and *Phoronis psammophila* is either dioecious or protandric. *Phoronopsis harmeri* (14), *Phoronis mulleri* (17), *Phoronis psammophila* (6), and *Phoronis architecta* (2) shed ova of small size, which develop through a relatively long pelagic larval phase, known thoroughly only for *P. mulleri* (17), a feature which may possibly be characteristic of this group.

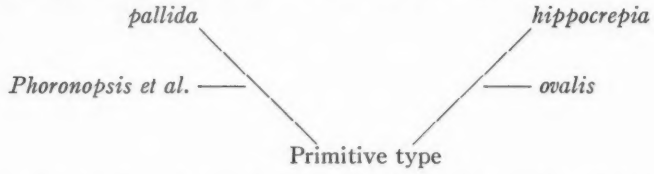
In the case of *Phoronis psammophila* and *Phoronopsis albomaculata* (17) specialized brooding habits are indicated, but inadequately described. The group appears to be both littoral and sublittoral in distribution.

Of the 18 species quoted earlier, there remain 2, *Phoronis pacifica* and *Phoronis buskii*, which are difficult to place because of lack of information. The brief descriptions of these two species leave this author with the feeling that the animals concerned most probably belong in the fourth category, but such a conclusion is necessarily based largely on negative evidence. *Phoronis pacifica* (20) is probably dioecious (only male individuals found), lives in straight tubes, has two giant fibers of unequal length and probably large diameter (judging from Fig. 2 in Torrey's paper). No longitudinal muscle formula is given. For *Phoronis buskii* there is even less information available. The natural habit of the animal is not described, no mention is made of giant nerve fibers, and the species is considered to be probably hermaphrodite, although the text of the description suggests a female gonad plus associated vasoperitoneal tissue rather than an hermaphrodite organ. MacIntosh's (11) conclusions are fairly obviously influenced by earlier accounts of *Phoronis hippocrepia*, which further compounds the difficulty of assessing the real nature of this phoronid from the Philippines. Obviously, until *P. pacifica* and *P. buskii* are collected and studied again there is little sense in trying to relate them to other, better known species.

It must be emphasized that this study, based largely on preserved material collected by others and sent to the author, is by no means exhaustive. The taxonomy of the Phoronidea obviously needs revision and it is clear that studies directed toward this end should be based on populations collected carefully for a period of a year or more. This investigation has shown that a considerable degree of morphological variation may be expected within a population and this should serve to point out the care which must be exercised in using such morphological features in the diagnosis of species. The suggestion of Silen (15) that the phylum Phoronidea can most naturally be divided into a few major taxonomic categories is supported by this study of North American forms and it is clear that the establishment of such categories will involve a number of nomenclatural changes. The investigation of North American forms, incomplete as it is, has served to emphasize the probable natural subdivision of the phylum into a few major categories and has made possible an estimation of the more important distinguishing features of each group. Three of these groups, represented by *P. ovalis*, *P. pallida*, and *P. hippocrepia*, appear fairly clear-cut already. The fourth category, as designated here, is less sharply defined, differs from that suggested by Silen, and it is here that more studies are most necessary. It seems unwise, therefore, to pursue the taxonomic designation and composition (in terms of species) of these categories until more information is available concerning the morphology and life histories of the animals involved.

The evolutionary implication of this study in phoronid taxonomy is of some interest. The cosmopolitan nature of each of the four categories

suggested here would seem to indicate an early diversification of the primitive phoronid type into four new types differing from one another anatomically and to some extent ecologically. Following this initial diversification, only relatively minor changes in anatomy and living habits seem to have accompanied the geographic radiation of the new types. Of the four groups postulated as derived from the primitive type, *P. ovalis* is probably closest to the primitive type with regard to adult morphology, although the reproductive habits of *ovalis* are highly specialized. The *P. hippocrepia* group represents a type morphologically more complex than *P. ovalis*, the two groups sharing to some extent the limestone boring habit. The *P. pallida* and the *Phoronopsis et al.* groups are probably more closely related to one another than they are to the other two categories. Both live in vertical tubes in a mud–sand substrate, both shed ova of small size, and both have a giant fiber of large diameter. This possible scheme of relationship between the four basic phoronid types is shown in the diagram below.




Apart from the relationships between the various phronid types, there is scope for interesting study and speculation concerning the importance in the geographic dispersal of these groups of factors such as egg size, brooding habits, planktonic larval life, the settling requirements of the larva, and asexual modes of reproduction. In conclusion, therefore, there is clearly a need for long-term studies on living animals from several localities, similar to the work of Silen (15, 16, and 17) on phoronids from the Gullmar Fiord area, before taxonomic and phylogenetic relationships within this group can be determined with any degree of confidence.

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# HISTOCHEMICAL STUDIES ON LOCALIZATION AND DISTRIBUTION OF ESTERASES IN THE SALIVARY GLANDS OF THE LARGE MILKWEED BUG, ONCOPELTUS FASCIATUS (DALL.) (HEMIPTERA:LYGAEIDAE)[1]

E. H. SALKELD[2]

## Abstract

The posterior lobe of the salivary glands of the large milkweed bug, *Oncopeltus fasciatus* (Dall.), was rich in a nonspecific esterase. An esterase was also localized in the epidermal cells of the tracheae and body wall and in the epithelial cells of the first part of the mid-gut. No true lipase was found in the salivary glands or in the head or thorax.

## Introduction

Bronskill *et al.* (2) have shown that the various lobes of the salivary glands of the large milkweed bug, *Oncopeltus fasciatus* (Dall.), contain different digestive enzymes and have demonstrated the presence of four. One of these, localized in the posterior lobe, was identified as a lipase by a histochemical technique in which Tween 60 was used as the substrate. Gomori (7) and Nachlas and Seligman (11) have shown that this technique is not specific for lipase but also indicates the presence of nonspecific esterases. Accordingly, a histochemical study of the salivary glands of *O. fasciatus* was undertaken to determine the nature of the enzyme present in the posterior lobe and to investigate the types and distributions of other esterases that might be present in the glands.

## Methods

Salivary glands obtained from newly molted, feeding adults reared in the laboratory on milkweed seed and water were used, and occasionally glands from nymphs of all five instars. Gomori's method with Tween 60 (6) and the azo dye method with $\alpha$-naphthyl acetate (8,10) and naphthol AS acetate (8) as substrates were used with slight modifications to determine the presence and distributions of esterases; Gomori's method with Tween 80 (7) was used for the lipase determination. Whole, fresh glands or entire insects were fixed in acetone at 4° C for 24 hours, cleared in cedarwood oil for 12 hours, and placed in xylol for 30 minutes. The glands were transferred to a 1:1 mixture of xylol and paraffin (m.p. 52–54° C) in a 37° C oven for 30 minutes, then placed in fresh paraffin at 56° C, and held under vacuum at 15 mm pressure for 30 minutes. They were embedded, sectioned at 7$\mu$, mounted on albumenized slides, and dried. The sections were coated with paraffin by placing the slides in the oven at 56° C for 5 minutes, and stored at 4° C until required.

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For use, the slides were carried through xylol and graded acetone–water mixtures to water in the azo dye techniques and through graded alcohol–water mixtures in the two Tween tests.

*Azo Dye Method*

*α-Naphthyl acetate.*—Slides were incubated in the following substrate mixture at room temperature for 20 minutes: 1% solution of α-naphthyl acetate[3] in 50% acetone, 1 cc; naphthanil diazo blue B[4], 40 mg; 2 *M* sodium chloride, 50 cc; 0.1 *M* barbiturate buffer of pH 7.8, 20 cc; distilled water, 29 cc.

*Naphthol AS acetate.*—Slides were incubated in the following substrate mixture at room temperature for 2 hours: 1% solution of naphthol AS acetate[5] in 10 cc of a 1:1 mixture of acetone and propylene glycol, 1 cc; naphthanil diazo red RC[6], 40 mg; 0.2 *M* phosphate buffer of pH 7.1, 5 cc; propylene glycol, 10 cc; distilled water, 40 cc.

The incubation media were prepared and filtered immediately before use; the substrate was omitted from the medium for the control sections. After incubation, the slides were washed in water and mounted in glycerine jelly.

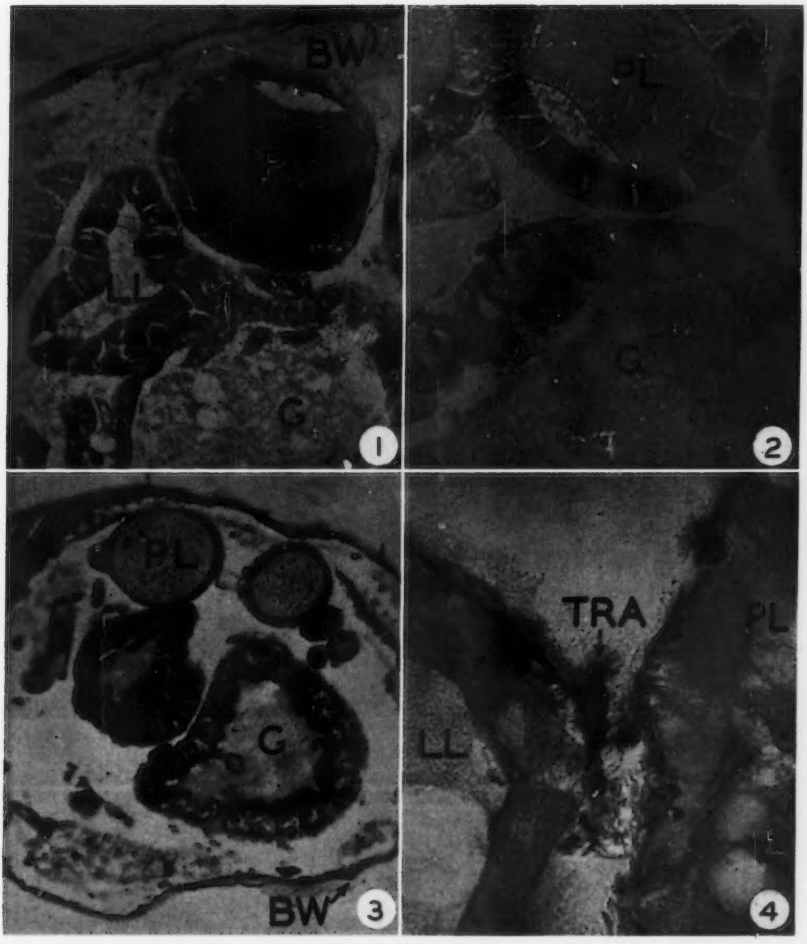
*Methods with Tween 60 and Tween 80*

Slides were incubated in the following substrate mixture for 12 hours at 37° C: 2% solution of Tween 60[7] or Tween 80[7] in water, 5 cc; 0.1 *M* tris (hydroxymethyl)aminomethane-HCl buffer of pH 7.3, 20 cc; distilled water, 20 cc; 2% anhydrous calcium chloride, 5 cc. Before being incubated, the control slides were soaked in 5% phenol for 2 minutes at room temperature to inactivate the enzyme. After incubation, all slides were rinsed in distilled water and treated with a 2% solution of lead nitrate for 10 minutes. They were washed thoroughly, treated for 2 minutes in a 1:100 dilution of yellow ammonium sulphide in water, washed thoroughly again, and counterstained in an aqueous solution of 1% eosin. After dehydration, they were cleared in dichloroethylene and mounted in Canada balsam dissolved in dichloroethylene.

## Results and Discussion

There were some slight differences in localization of esterase activity when the different substrates were used. Pronounced activity always occurred in the contents of the posterior lobe with the substrates Tween 60 (Fig. 1) and naphthol AS acetate (Fig. 2), and weak activity was indicated here by α-naphthyl acetate (Fig. 3). The cytoplasm of the epithelial cells of this lobe was negative with all substrates except for an occasional positive reaction with Tween 60; this may have been the result of diffusion of the enzyme from the lumen (9). Positive esterase activity, demonstrated by all three substrates, and especially by Tween 60 and α-naphthyl acetate, occurred in the

[3]Eastman Organic Chemicals, Rochester 3, N. Y.
[4]Tetrazotized *o*-dianisidine, Dajac Laboratories, The Borden Company, Philadelphia 24, Pa.
[5]Dajac Laboratories, The Borden Company, Philadelphia 24, Pa.
[6]Diazotized 2-amino-4-chloroanisole, Dajac Laboratories, The Borden Company, Philadelphia 24, Pa.
[7]Brickman and Company, Montreal, Canada.




FIGS. 1 to 3. Cross section of thorax of *O. fasciatus* showing localization of esterases. FIG. 1, in posterior lobe of salivary gland by Tween 60. ×60. FIG. 2, in epithelial cells of gut by naphthol AS acetate. ×336. FIG. 3, in epithelial cells of gut by α-naphthyl acetate. ×29.

FIG. 4. Cross section of lateral and posterior lobes of salivary gland: localization of esterase in trachea by α-naphthyl acetate. ×240.

ABBREVIATIONS: BW, body wall; G, gut; LL, lateral lobe; PL, posterior lobe; TRA, trachea.

epidermal cells of the numerous tracheae of the salivary glands (Fig. 4) and of tracheae in other parts of the head and thorax.

A positive reaction with Tween 60 was noticed in the dermal cells of the body wall and in the wing buds of the various nymphal instars; only a faint reaction occurred with the other two substrates. In addition, a strikingly positive reaction occurred in the free cell border of the epithelium in the forepart of the mid-gut with $\alpha$-naphthyl acetate and naphthol AS acetate (Figs. 2, 3) but only occasional activity occurred with Tween 60.

The existence of several esterase systems, characterized by their specificity to different substrates, has been suggested in mammalian tissue (3, 11) and in some tissues of the amphibian *Necturus maculosus* Rafinesque (5). However, the slight variations in the sites of esterase activity noted when three different substrates were tested on tissues of *O. fasciatus* do not appear to warrant such a suggestion. The presence of an esterase in the posterior lobe of the salivary gland and in the gut of this insect indicates that it may have a digestive function. It seems unlikely, however, that such a function is served by the esterase in the dermal cells and in the epithelial cells of the tracheae. Day (4), using $\beta$-naphthyl acetate as substrate, found a non-specific esterase in low concentration in the ducts of the salivary glands of the cockroach *Periplaneta americana* (L.) but could not demonstrate it in the cells of the acini. He was unable to define its function.

With Tween 80 as substrate, no reaction was observed in any part of the salivary glands or in the head or thorax of the insect, indicating the absence of true (7) lipase. Baptist (1) and Nuorteva (12) have reported the presence of lipase in the salivary glands of certain other Heteroptera. It would be of interest to determine whether any of these insects have a true lipase.

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# A MICROSPORIDIAN INFECTION IN THE JACK-PINE BUDWORM, CHORISTONEURA PINUS FREE.[1]

H. M. THOMSON[2]


## Abstract

A microsporidian parasite of the jack-pine budworm is described and found to be very similar to *Perezia fumiferanae* Thom., which attacks the spruce budworm. The differences between the two species of Microsporidia are not considered great enough to justify the creation of a new species at the present time; however, with the continued isolation of the host species, the differences may become greater.


In a previous paper (4) it was shown that the microsporidian parasite *Perezia fumiferanae* Thom. of the spruce budworm, *Choristoneura fumiferana* (Clem.), was also able to infect the jack-pine budworm, *C. pinus* Free. Although this cross infection had been carried out under laboratory conditions, only one jack-pine budworm naturally infected by a microsporidian had been found by the Insect Disease Survey of the Canadian Department of Agriculture since its inception in 1952. Recently two populations of jack-pine budworm, one in Ontario and one in Manitoba, have yielded individuals infected by a microsporidian. The purpose of this paper is to describe this organism and compare it with *P. fumiferanae*, the spruce budworm microsporidian.

## Methods and Materials

The life cycle of the jack-pine budworm microsporidian was determined by examining smears of infected individuals, fixed in May-Grünwald solution and stained by Giemsa's stain. The cycle was derived by arranging the observed stages in a logical order based on present knowledge of cell division, aided whenever possible by the observed chronological sequence of the various stages.

The sites of infection in the host larvae were determined by sectioning infected larvae and staining them with Heidenhain's haematoxylin and orange G. The spores in the tissues retained the haematoxylin stain longer than the host tissues upon differentiation with 2% iron alum.

The length of the polar filament was measured after ejection was accomplished by flooding spores, dried on a slide, with a commercial preparation of liver extract. Only a small proportion of spores ejected their filaments. Ejection of the filament could also be accomplished by the application of 3% hydrogen peroxide, but the release of oxygen caused great turbulence under the cover slip making measurement difficult. The filaments were observed by phase microscopy.

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## Life Cycle

*Schizogony*

The earliest schizonts observed are composed of two small, compact, dark-staining nuclei surrounded by a clear narrow band of cytoplasm. This is apparently a rapidly dividing stage as many of these schizonts were seen to be undergoing cell division (Fig. 1*a*). Growth and multiplication occur in the host cell cytoplasm, the schizonts getting larger with greater amounts of cytoplasm and the nuclei becoming vacuolated and less deeply stained. Binucleate and tetranucleate schizonts are most commonly observed (Figs. 1*b*,*c*), but occasionally chain-forms of up to four cells have been seen (Fig. 1*d*).

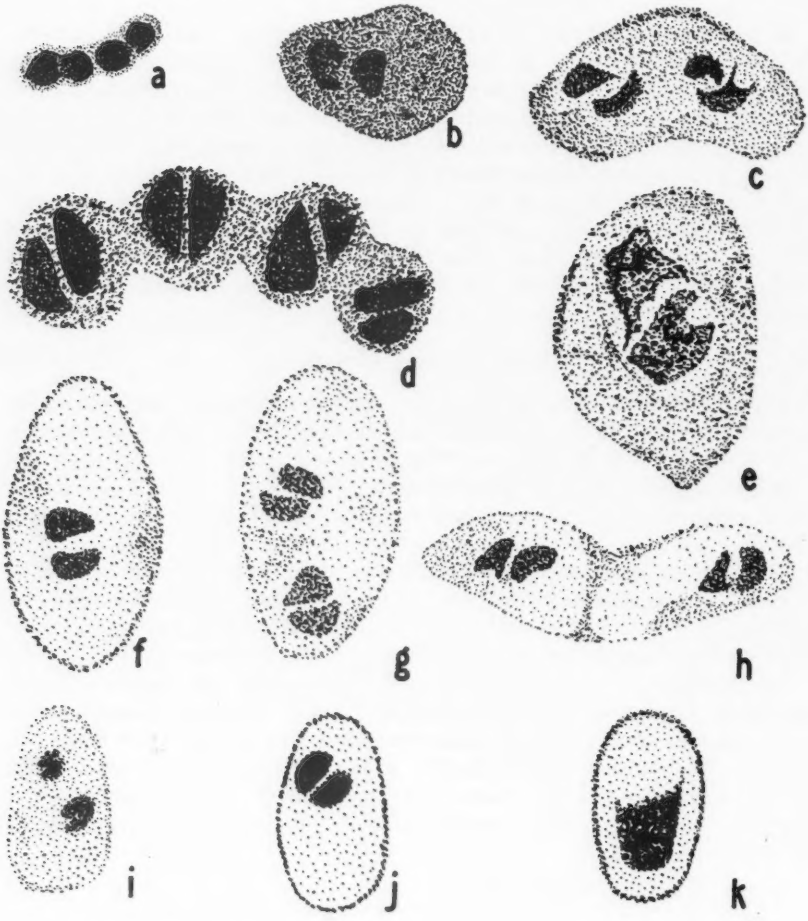


FIG. 1. Stages in the life cycle of the jack-pine budworm microsporidian. ×7200.

PLATE I

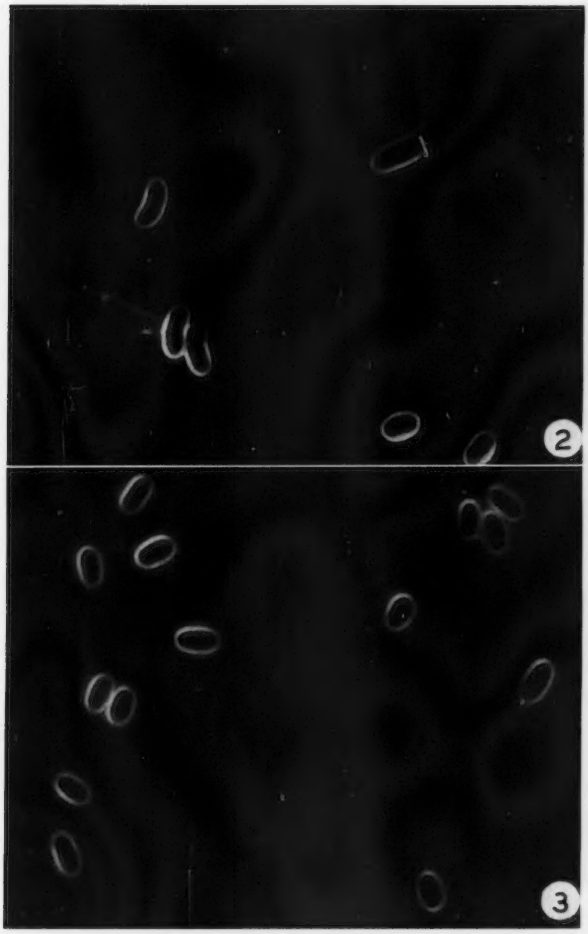


FIG. 2. Living spores of the spruce budworm microsporidian. ×1800.
FIG. 3. Living spores of the jack-pine budworm microsporidian. ×1800.

Multinucleate schizonts as described for the spruce budworm microsporidian have not been observed. The largest schizonts measure approximately 5 $\mu$ in diameter (Fig. 1*e*).

*Sporogony*

The largest binucleate schizonts apparently transform into oval-shaped sporonts with two dark-staining nuclei (Fig. 1*f*), in which the cytoplasm is vacuolated and stains weakly. The two nuclei become four, and two migrate to each end of the sporont (Fig. 1*g*), which takes on a characteristic bilobed appearance (Fig. 1*h*), and eventually divides into two sporoblasts (Fig. 1*i*). The sporoblasts shrink, the cytoplasm no longer stains, and a spore wall appears. The young spores at first contain two distinct nuclei (Fig. 1*j*) which later apparently fuse into an irregularly-shaped mass (Fig. 1*k*).

Living spores are highly refractive and measure 2×3–5 $\mu$. Some are straight-sided, but in general the sides bulge slightly. The polar filament varies from 60 to 100 $\mu$ in length.

## Pathology

Examination of stained sections of infected larvae shows that the microsporidian develops in a variety of host tissues. The mid-gut cells are heavily infected, and as the development of the parasite is most advanced in this tissue it would seem to be the primary site of infection. The parasite is also found in the Malpighian tubules, silk glands, fat body, integument, and male and female gonads.

## Discussion

The description of the jack-pine budworm microsporidian is similar in most respects to that of the spruce budworm microsporidian (3). The shape and sizes of the stages in the life cycles, the length of the polar filament, and the host tissues attacked are very similar. The main difference is the rather common occurrence of kidney-shaped spores in the spruce budworm microsporidian (Fig. 2) and their relative absence in the jack-pine budworm microsporidian (Fig. 3). Another difference is the presence of schizonts containing as many as 12 nuclei among the spruce budworm microsporidian whereas the jack-pine budworm microsporidian has not been observed to contain more than 4. It is not believed that these differences are sufficient to create a separate species of the jack-pine budworm microsporidian.

The spruce budworm and the jack-pine budworm have only recently been separated into different species (1). While under laboratory conditions they are able to interbreed to a considerable extent, they are prevented from doing so in nature by temporal and ecological factors (2). It is not unreasonable to believe that as the respective hosts became isolated, variations appeared in what was once the common microsporidian parasite. In time these variations may develop into true specific differences.

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# ON THE TREMATODES BRACHYLECITHUM ORFI SP. NOV. (DICROCOELIIDAE) AND TANAISIA SP. (EUCOTYLIDAE) FROM THE RUFFED GROUSE, BONASA UMBELLUS L.[1]

NEWTON KINGSTON[2] AND REINO S. FREEMAN[3]

## Abstract

A new species of dicrocoeliid trematode, *Brachylecithum orfi* sp. nov., is reported and described from the biliary ducts of the ruffed grouse, *Bonasa umbellus* L., from Ontario and Michigan. *Tanaisia* sp. also is reported from this host. The incidence of these flukes is discussed.

## Introduction

A dicrocoeliid trematode has been commonly found in the biliary ducts of ruffed grouse examined over the past several years from Ontario. The same parasite has been taken in Michigan from this host by Cowan and Spaulding (12), who have kindly let us examine their specimens. As the specimens from Ontario and Michigan differ from other species of dicrocoeliids, we have assigned them to a new species in the genus *Brachylecithum* here described as *Brachylecithum orfi* sp. nov.

A species of the genus *Tanaisia* Skrjabin, 1924, as emended by Byrd and Denton (2), also has been found in the ruffed grouse in the urinary tubules.

## Description

*Genus: Brachylecithum* Shtrom, 1940, as emended by Skrjabin and Evranova (10).

*Brachylecithum orfi* sp. nov.[4]

Body of mature specimen elongate-cylindrical with nearly parallel sides, tapering gradually in posttesticular region; body length 9.9 (4.4–11.2), body width 0.33 (0.21–0.43) in region of anterior testis. Ratio of body width to body length 1:30 (1:16–38). Cuticle aspinose, without tuberculations. Oral sucker elongate-oval 0.29 in length by 0.24 in width (0.25–0.43 by 0.23–0.40), subterminal, with a dorsal, overhanging lip (seen best in sectioned material), mouth opening ventrally. Acetabulum without auricles, transversely oval 0.29 in length by 0.32 in width (0.26–0.49 by 0.31–0.43), anterior margin 0.73 from anterior end of worm (0.45–1.13), cavity (usually) not obliterated by protruding center. Ratio of oral sucker to acetabulum 1:1 (1:0.92–1.27). Prepharynx absent. Pharynx pyriform, wider than long (generally), 0.070 in length by 0.086 in width (0.057–0.095 by 0.067–0.112).

[1]Manuscript received December 11, 1958.
Contribution from the University of Toronto and the Ontario Research Foundation, Toronto, Ontario.
[2]School of Hygiene, University of Toronto.
[3]Ontario Research Foundation.
[4]All measurements in mm; data outside parentheses from holotype from Ontario; data in parentheses from 22 paratypes from 8 ruffed grouse from Ontario and 3 paratypes from Michigan.

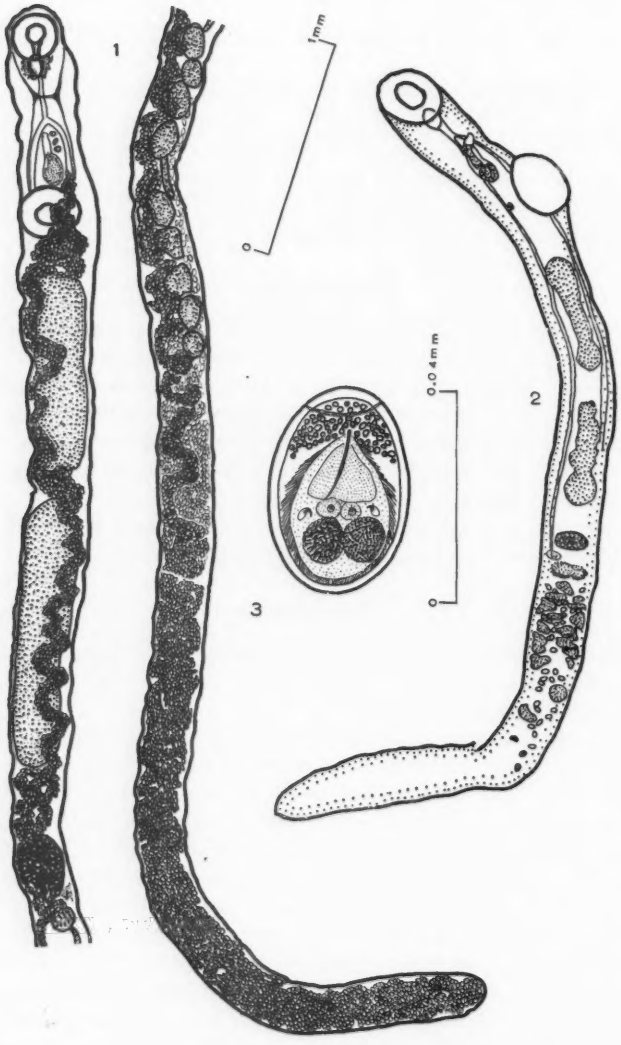


FIG. 1. *Brachylecithum orfi* sp. nov. Holotype. Anterior portion of body in ventral view, posterior part of body lateral owing to rotation when mounting. The figure is divided for convenience of reproduction. The holotype is entire. 9.9 mm. FIG. 2. *Brachylecithum orfi* sp. nov. Paratype. Ventrolateral view, 4.4 mm. Specimen presumed immature on basis of size and number of eggs in uterine coils. FIG. 3. Egg of *Brachylecithum orfi* sp. nov. drawn from unfixed material. Composite. All drawings made with the aid of a microprojector or the camera lucida.

Oesophagus narrow, thin-walled, slightly undulating, 0.14 in length by 0.025 in width (0.14–0.21 by 0.025–0.047), bifurcating immediately anterior to female genital pore. Caeca paired, narrow, thin-walled, difficult to follow posterior to testes, extent not determined. Male genital pore median, opening between oral sucker and acetabulum 0.51 from anterior end of worm (0.36 in contracted specimen to 0.73 in large extended specimen). Cirrus sac 0.30 in length by 0.11 in width (0.25–0.41 by 0.08–0.12), containing an unarmed, eversible cirrus and a coiled seminal vesicle; bulbous portion of cirrus sac posterior and dorsal to anterior margin of acetabulum. Testes elongate-cylindrical, tandem, almost filling body width, with smooth margins (or only slightly indented; in some specimens, presumed young, testes may (Fig. 2) be lobate), anterior testis (generally) shorter in length than posterior testis: anterior testis 0.96 in length by 0.25 in width (0.30–1.23 by 0.11–0.35), posterior testis 1.29 in length by 0.26 in width (0.45–1.63 by 0.10–0.30); anterior testis begins 1.13 from anterior end of worm (0.80–1.60), posterior testis begins 2.23 from anterior end of worm (1.20–2.80). Space between testes 0.13 (0.00–0.15) occupied (or not) by loop(s) of uterus. Ratio of length of testes field[5] to body length 1:4.3 (1:3.33–5.23). Ovary dorsal, behind posterior margin of posterior testis, (round to) oval, 0.28 in length by 0.19 in width (0.10–0.35 by 0.08–0.25), located 3.81 from anterior end of worm (1.96–4.50). Seminal receptacle globular, 0.12 in length by 0.11 in width (0.11–0.12 by 0.07–0.11), anterior margin 0.05 (0.01–0.05) behind and dorsal to the posterior border of ovary. Mehlis' gland posterior to seminal receptacle. Laurer's canal not seen. Vitellaria (Figs. 1 and 2) entirely posterior to ovary, and seminal receptacle consisting of 10 (6–12) large irregular follicles on each side (fields frequently joined anteriorly; number of follicles on one side may vary from number on other), occupying a total field of 1.50 in length (0.60–1.50) beginning 4.36 from anterior end of worm (2.48–5.05). Descending limb of uterus, on dorsal side of body, containing developing eggs, leaves the ovary, passes in coils between vitellaria to within 0.05 (0.070) of posterior end of body where it turns and ascends, on ventral side of body, in wide loops to the region of the vitellaria. Posterior to the vitellaria, approximately 6.60 from the anterior end of the body, the ascending uterus contains dark, fully embryonated eggs; it continues in coils ventral to vitelline follicles, passes with compressed loop(s) (one to six) between vitellaria and ovary, proceeds ventral (or lateral) to ovary, with four loops (two to four) between anterior margin of ovary and posterior margin of posterior testis, (generally) runs ventral to posterior testis, with one loop (none to two) between posterior testis and anterior testis, then in slight sinuosities ventrolateral to anterior testis (sometimes ventral or dorsal) to region behind acetabulum where it passes in several coils (three to seven) to female genital pore located median and immediately anterior to male genital pore. Excretory pore posterior, terminal. Fully developed ova dark brown, thick-shelled, operculate (in

[5]The space measured from the anterior margin of the anterior testis to the posterior margin of the posterior testis.

unfixed material a fully developed miracidium possessing a stylet and two large, granular, oval vesicles can be seen; these ova range in size from 0.037–0.047 by 0.025–0.032) (Fig. 3).

*Host: Bonasa umbellus* L.

*Habitat:* Biliary ducts.

*Locality:* Ontario—Algonquin Park (holotype) and Big Island, Lake of the Woods; Michigan—Ottawa National Forest.

*Molluscan hosts: Zonitoides arboreus, Zonitoides nitidus, Deroceras reticulatum, Deroceras laeve, Cionella lubrica.*[6]

*Type specimen:* Holotype and nine paratypes in U.S.N.M. Helminthological Collection, Nos. 38391 and 38392 respectively.

Twenty-six unfixed worms from three ruffed grouse from Algonquin Park measured 6.9–15.0 mm in length. Width measurements of worms which were 9.4–13.0 mm in length ranged from 0.44–0.55 mm. The range of the ratios of the body widths to the body lengths, 1:20–23.

Some features of these worms were more apparent in unfixed specimens than in fixed material: namely, the subterminal position of the oral sucker; the nature and extent of the vitelline follicles; the occluded cavity of the acetabulum in some specimens; and the gravid condition of the uterus. The identity of *Brachylecithum orfi* can be confirmed in unfixed material by noting that the testes field to body length ratio lies in the range of 1:3–5.

## Discussion

*Brachylecithum orfi* sp. nov. differs from the other species now contained in the genus in the following characters: (1) by its greater length, (2) by its larger ratio of body width to body length, (3) by its longer testes, (4) by its smaller ratio of testes field to body length, and (5) by the extent and position of the vitelline field, which lies in whole, or in large part, in the posterior half of the body.

*Brachylecithum orfi* while resembling *Brachylecithum eugenia* Oschmarin, 1947 (in Skrjabin and Evranova (10)) differs from it as follows: (1) The testes of *B. orfi* are nearly twice the length of those in the latter species; some specimens of *B. orfi* and *B. eugenia* have testes of comparable length, but the body lengths for such specimens of *B. orfi* are much shorter than is the body length of *B. eugenia*, and the ratio of the testes field to body length remains near 1:4 for *B. orfi* while this ratio is 1:9 for *B. eugenia*. (2) Further, *B. orfi* lacks auricles on the acetabulum which *B. eugenia* possesses. (3) Finally, the length of the vitelline field of *B. orfi* is greater than that of *B. eugenia*.

*Brachylecithum orfi* although similar to *Brachylecithum papabejani* (Skrjabin and Udinzew, 1930) Shtrom, 1940 differs from it as follows: (1) *B. orfi* is

[6]Determined by feeding experiments, details of which will be given in another paper.

narrower thus possessing a greater ratio of body width to body length than does *B. papabejani* (1:10.8–11.2). (2) The eggs of *B. orfi* are larger than those of *B. papabejani.*[7]

*Brachylecithum orfi* somewhat resembles *Brachylecithum gruis* Denton and Byrd, 1951. It differs from *B. gruis* in that while both species have elongate testes, in only one short paratype specimen of *B. orfi* are the lengths of the testes comparable to those of *B. gruis;* further, the margins of the testes are smooth in mature specimens of *B. orfi* while they are lobate in *B. gruis.* The size of the oral and ventral suckers is greater in *B. orfi* than that given for *B. gruis.* The vitelline field in *B. orfi* is up to four times longer than in *B. gruis.* The vitelline field of *B. gruis* apparently lies wholly in the anterior half of the body whereas in specimens of *B. orfi* the vitelline field always extends into, or lies wholly within, the posterior half of the body.

Ishii (6) and Erickson *et al.* (4) reported finding a dicrocoeliid identified as *Lutztrema* (=*Lyperosomum*) *monenteron* (Price & McIntosh, 1935) Travassos, 1941 in ruffed grouse in Minnesota. Erickson *et al.* gave no description, and Ishii's description is inadequate for generic or species identification. Denton and Byrd (3) examined Ishii's slides and concluded that these specimens must remain as *species inquirenda* pending study of more favorable material.

Through the kindness of Drs. A. B. Erickson, Minnesota Department of Conservation, and E. F. Cook, University of Minnesota, we examined the material recovered by Dr. Erickson from a ruffed grouse. From the condition of the specimen we cannot state with exactness what species this is, but the oral and ventral suckers are approximately equal in size, which character more nearly approaches that in *Brachylecithum* than *Lutztrema.* The intestinal caeca bifurcate at the level of the genital pore, which character removes it from the genus *Lutztrema* sensu Travassos (13). Only a few large follicles remain of the vitellaria situated across the breadth of the body, a character common to *Brachylecithum* (9, 10) and *Lutztrema* (3, 10, 13) and in contrast to the finer follicles arranged along the lateral margins of the body of *Lyperosomum* (3, 10, 14).

It appears most probable, therefore, that Erickson's specimen belongs in the genus *Brachylecithum* rather than in the genus to which he assigned it.

## Time and Incidence of Infection

A total of 87 ruffed grouse, of which 44 harbored *B. orfi*, were examined from Algonquin Park and Big Island, Lake of the Woods, Ontario, between 1952 and 1958 (Table I). Twenty-two of these were young birds, examined

[7]The yolk glands in *B. papabejani* are described as comprising 0.146–0.165 mm of the length of the body, but the illustrations in both works show that the yolk glands occupy approximately 1.45 to 1.65 mm of the length of the body. Apparently a simple decimal error was made in the original description (11) which was repeated without comment in the subsequent review by Skrjabin and Evranova (10) and by Travassos in his revision of the Dicrocoeliidae (14). The measurements of the yolk glands of *B. papabejani* thus fall within the range of those found in *B. orfi.*

July through October during 1955 to 1957, and 7 harbored *B. orfi*; Cowan and Spaulding (12) found 14 of 17 adult birds and 3 of 40 juveniles 9 to 13 weeks of age infected in Michigan in 1957.

The earliest infections in juveniles in Ontario appeared toward the last of August in both years, when the birds were 8 to 10 weeks of age (1) (Table I, footnotes). This does not indicate that infections are acquired only by young birds, however, since one adult bird examined in early July, 1956, harbored young worms, judging from their size and egg content. If *B. orfi* has a life cycle like the other dicrocoeliids for which the pattern (7, 8) is known, the flukes reach sexual maturity in 1 to 2 months following the ingestion of metacercariae by the bird. Thus, the adult bird killed in July must have been exposed to infection in June or possibly as early as May. Young birds are largely insectivorous in the early weeks of life (1) and it is likely they are exposed most frequently at this time. Since but 32% of juvenile birds were found to be infected as compared with 55% of adult birds, it is apparent that infection can be acquired after the bird has reached maturity although most probably the greater number of ruffed grouse become infected in their first months of life.

TABLE I

Infections with *Brachylecithum orfi* sp. nov. in ruffed grouse by age and sex (Ontario records)

| Year (no. exam.) | Number examined/number infected | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Adult male | Juv. male | Adult female | Juv. female | Ad. ? | Juv. ? | ? ? | Total |
| 1952 ( 5) | 2/0 | | 3/1 | | | | | |
| 1953 (10) | 4/1 | | 6/5 | | | | | |
| 1955 ( 1) | | | | | | 1/0[1] | | |
| 1956 (18) | 4/3 | 1/1[2] | 4/4 | | | 5/0[3] | 4/4 | |
| 1957 (45) | 11/5 | 5/4[4] | 14/9 | 5/1[5] | 4/2 | 5/1[6] | 1/0 | |
| 1958 ( 8) | 5/2 | | 3/1 | | | | | |
| Total | 26/11 | 6/5 | 30/20 | 5/1 | 4/2 | 11/1 | 5/4 | 87/44 |
| Total males | 32/16 | | | | | | | |
| Total females | 35/21 | | | | | | | |
| Total adults | 60/33 | | | | | | | |
| Total juvs. | 22/7 | | | | | | | |

Algonquin Park: [1]15 VIII; [2]20 VIII; [3]12 VII—3 VIII; [4]31 VIII—6 IX; [5]3 IX—2 X; [6]3 IX—2 X.

## *Tanaisia* sp.

*Tanaisia* sp. occurred in 8 of 77 birds examined from Algonquin Park. Cowan and Spaulding (12) also report the presence of this parasite in Michigan. As there is some confusion in the taxonomy within this genus (2, 5), our material has not been assigned to any species pending further study.

## Acknowledgments

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# SPECIFIC INHIBITION OF DIFFERENTIATION IN THE FROG EMBRYO BY CELL-FREE HOMOGENATES OF ADULT TISSUES[1]

R. B. CLARKE[2] AND D. J. MCCALLION

## Abstract

The hypothesis that adult tissues produce substances which may specifically inhibit the differentiation of like embryonic tissues and organs has been tested by Rose (3) by rearing frog embryos in the presence of living fragments of adult organs. In the present investigation frog embryos were reared in media containing cell-free homogenates of adult brain or heart. The homogenates specifically suppressed or inhibited the development of these organs in about 10–15% of the embryos tested.

## Introduction

The concept of organ-specific control of growth is not a new one and has appeared, over the years, in many publications. Recently, Rose (2) has advanced the hypothesis that differentiation proceeds as more rapidly developing regions suppress like development in more slowly developing adjacent areas. Thus, the slower regions, prevented from forming structures already differentiating, are channelled into other kinds of differentiation and thereby complete the pattern of the whole through the action of a hierarchy of self-limiting reactions. In the past few years, Rose (3, 4, 5) has continued in his researches to seek a critical test of the hypothesis and to attempt to evaluate its broad biological application. In his studies on the frog embryo he has investigated the effects of blood and of fragments of living brain and heart tissue on the development of these organs in the embryo. The results of his investigations suggest that older, well-differentiated tissues inhibit specifically the differentiation of homologous embryonic tissues. Rose (4) believed that inhibition was effected by products of the *living* tissues. The present paper reports the results of an investigation of the effects of cell-free homogenates of adult frog brain and heart on the development of these organs in the frog embryo.

## Materials and Methods

The frog eggs used in these experiments were obtained from healthy adult *Rana pipiens* females by induced ovulation and artificial fertilization after the method of Rugh (6). Cell-free homogenates of adult frog cerebrum (pituitary excluded) and heart were prepared by grinding in a mortar with washed sterile sand. Suspensions of these homogenates in chlorine-free tap water were used as culture media. Developing eggs were reared in small groups in 50 cc of culture medium in 11-cm finger bowls at 21°–23° C. The suspensions were changed at 12-hour intervals for a period from 36 to 84

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[2]Holder of a National Research Council Studentship during 1957–58.

hours. Eggs were first exposed to these suspensions following fertilization or at yolk plug stages. Thus, eggs were treated from cleavage to gastrulation, from yolk plug through neurulation, or throughout the entire period from cleavage through neurulation. Following treatment the eggs were transferred to fresh tap water. Final observations of the embryos were made on the sixth day following fertilization. For microscopic study, embryos were fixed in Smith's fluid, double embedded, sectioned at 10 $\mu$, and stained with hematoxylin and eosin (H and E).

## Results of Experiments

The effects of the treatments used in these experiments were often quite varied. There was, generally, a lag in the rate of development of the embryos as compared with normal control embryos. Many embryos showed gross general abnormalities, which made the identification of specific defects difficult. The general defects found in experimental embryos were not more frequent than similar defects found in control embryos cultured in tap water.

A total of 144 eggs were reared in suspensions of homogenates of three frog forebrains from beginning cleavage through yolk plug stages; 131 embryos survived, 10 of which were abnormal. None showed any specifically neural defects.

A total of 207 eggs were exposed to suspensions of three frog forebrains from early yolk plug stages to neural tube formation; 160 embryos survived, and of these, 25 showed specifically neural defects. Where development had been arrested at neural tube or tail bud stages the anterior medullary plate was suppressed or nearly absent or the neural tube was small and remained open anteriorly. The optic bulges were absent. In embryos that developed beyond tail bud stages the ventricles of the brain were very small or absent so that the brain was solid or thick-walled (Figs. 3, 4, 6, and 7). The telencephalon had only one ventricle (Fig. 1). Optic structures were rudimentary or absent (Figs. 3, 4). In some embryos the notochord was absent, or present only in the trunk.

A total of 105 eggs were reared in suspensions of homogenates of one chick cerebral hemisphere. Of these, 92 survived and 15 showed anterior end defects similar to those described above.

There were 124 eggs treated with suspensions of homogenates of three frog hearts from early cleavage to neural tube stages. These embryos showed no neural defects but many of them were edematous in the heart region, exhibiting what Rose (3) termed the "heartless syndrome".

Twelve such embryos were sectioned and stained. In two of these the heart was extremely small, while the neighboring organs had developed normally. In the others either slight abnormalities of the heart were associated with other abnormalities, or the heart was nearly normal.

Finally, eggs raised in suspensions of boiled brain homogenates all developed normally and were comparable to control embryos raised in tap water.

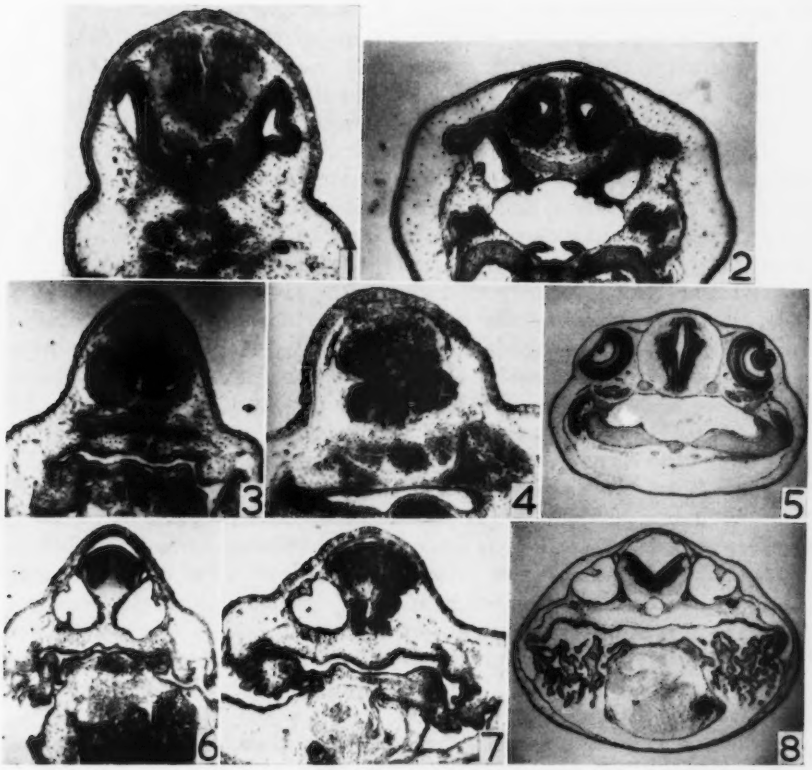


FIG. 1. Transverse section through the telencephalic region of an embryo treated with frog brain homogenate from yolk plug through neurulation. H and E. ×48. Note solid telencephalon and normal nasal choanae. Compare with Fig. 2.

FIG. 2. Transverse section through the telencephalic region of a normal 10-mm larva. H and E. ×35.

FIGS. 3 and 4 Transverse sections through the optic region of two embryos treated as the embryo in Fig. 1. H and E. ×28.5 and ×39.5 respectively. Note solid brain and rudimentary eyes. Compare with Fig. 5.

FIG. 5. Transverse section through the optic region of a normal 10-mm larva. H and E. ×16.5.

FIGS. 6 and 7 Transverse sections through the otic region of two embryos treated as the embryo in Fig. 1. H and E. ×34 and ×24 respectively. Note abnormalities of the brain and absence of notochord. The otic vesicles and cranial nerves are normal. Compare with Fig. 8.

FIG. 8. Transverse section through the otic region of a normal 10-mm larva. H and E. ×20.5.

## Discussion and Conclusions

The specific effects of cell-free homogenates of brain and heart on the development of these organs in the embryo are essentially similar to the effects of culturing embryos in the presence of living fragments of these organs (Rose (3)). Moreover, it is evident that the effect is not dependent upon the integrity of these tissues, since cell-free homogenates produce the same effects. Comparable results have also been obtained in regenerating planaria with breis of heads (Lender (1)).

The results of these experiments indicate with reasonable certainty that well-differentiated tissue liberates products which inhibit like differentiation in embryos and also that such products are present and active in cell-free homogenates. These products are probably thermolabile, since boiled tissue homogenates have no effect. Not only homogenates of frog brain but also chick brain homogenates inhibit neural differentiation. The inhibitory products would not, therefore, seem to be species-specific. The evidence, however, is not conclusive and this phenomenon requires further investigation.

Our results differ slightly from those of Rose (3), not in the kind or numbers of defects, but in the time at which treatment was effective. Rose found that if treatments were not begun until a late gastrula or neurula stage the eggs were not affected but developed on time and without defects. In our experiments eggs treated from cleavage to yolk plug only were not affected and all developed normally. Homogenates of brain tissue affected embryos only when eggs were treated, beginning at cleavage or yolk plug stages, through early neurula stages. Other than the nature of the treatments the only significant difference in the experiments was in the temperatures at which the experiments were conducted. Rose treated embryos at 9°–12° C whereas our experiments were carried out at 21°–23° C.

When fragments of tissues are used in treating embryos the possibility exists that the variations in numbers or types of defects may be due to variations in the concentrations of organs and tissues around the embryos. The use of homogenates excludes the possibility of variations in concentration of the effective substance. The small numbers of embryos affected and the variations in susceptibility of embryos to treatment remains unexplained.

We are also unable to explain adequately the suppression of notochord differentiation in the few cases where it occurred. This particular defect was apparently not seen in Rose's experiments.

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# SPECIFIC INHIBITION OF NEURAL DIFFERENTIATION IN THE CHICK EMBRYO[1]

R. B. CLARKE[2] AND D. J. MCCALLION

## Abstract

Suspensions in Locke's solution of cell-free homogenates of the cerebra of recently hatched chicks were injected into the yolk of chicken eggs of 20–24 hours of incubation. Eggs were similarly injected with homogenates of chick muscle, mouse brain, and mouse muscle. About 37% of the embryos exposed to chick brain homogenates showed some degree of inhibition of development of the brain. Other treatments did not produce this effect. This is presented as further evidence in support of the concept of specific inhibition as a developmental mechanism.

## Introduction

In a previous paper the authors (Clarke and McCallion (1)) reported the results of an investigation of the specific inhibitory effects of cell-free homogenates of adult frog organs on the differentiation of homologous organs in the frog embryo. The results of that investigation support the hypothesis of specific inhibition as a mechanism of development as advanced by Rose (5). The present paper reports the results of a similar investigation of the effects of cell-free homogenates on embryonic differentiation in the chick embryo.

## Materials and Methods

White leghorn eggs, obtained from a commercial hatchery, were used in these experiments. Cell-free homogenates of the cerebra of recently hatched white leghorn chicks were prepared under sterile conditions either in a Potter–Elvehjem homogenizer or by grinding in a mortar with washed sand. Homogenates were also prepared of mouse cerebrum and of chick and mouse muscle. Suspensions of these homogenates were prepared in sterile Locke's solution such that the ratio by volume, of original tissue to Locke's solution, was 1:4 to 1:6.

A sterile homogenate suspension (0.9 cc) was injected with a No. 20 needle into the yolk of the egg through a hole drilled in the blunt end of the egg shell. The hole in the shell was then sealed with paraffin. The eggs were injected at 20–24 hours of incubation and subsequently incubated for a further 24 hours. This method of injection takes advantage of the circulation of the yolk. Thus, the homogenate would be brought into contact with the developing

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[2]Holder of National Research Council Studentship during the year 1957–58.

embryo at about the stage of head-fold formation. As controls, eggs were injected with the same volume of sterile Locke's solution and otherwise treated in the same manner.

The embryos were removed from the eggs at the end of the incubation period and washed free of yolk. They were first examined unstained, and then, either as whole mounts stained with borax carmine, or serially sectioned and stained with hematoxylin and eosin (H and E).

## Results of Experiments

Of 115 eggs injected with suspensions of homogenates of chick cerebra, 42 embryos later showed some degree of inhibition of brain development only. The abnormalities of the brain in these embryos varied from a forebrain smaller than normal to a small, opaque club-shaped brain (Figs. 3, 4). Between these extremes were various degrees of forebrain deformity. Abnormalities were generally restricted to the forebrain.

Histological examination of representative abnormal embryos confirmed that the abnormalities were restricted to the brain. Other organs and tissues were normal. The forebrain was smaller and less well-differentiated than that of normal embryos of the same number of somites. The specific abnormalities of the forebrain were: very small ventricles with the walls of the forebrain thickened (Figs. 7, 8), partially open or poorly closed neural tube (Fig. 6), and optic vesicles reduced or absent.

Homogenates of chick muscle, mouse brain, or mouse muscle when injected into chicken eggs did not produce any specifically neural defects in the developing embryos.

The essential numerical results of all experiments are given in Table I.

A statistical analysis of these data was made in order to determine whether the occurrence of abnormalities of the brain alone in embryos treated with

TABLE I

| Treatment of eggs | Number of eggs treated | Number of embryos abnormal | Number of abnormalities of brain only |
|---|---|---|---|
| Untreated | 102 | 11 | 2 |
| Injected with mouse muscle homogenate | 10 | 0 | 0 |
| Injected with chick muscle homogenate | 19 | 1 | 0 |
| Injected with mouse brain homogenate | 13 | 3 | 0 |
| Injected with chick brain homogenate previously heated to 95° C | 14 | 3 | 1 |
| Injected with Locke's solution | 169 | 40 | 12 |
| Injected with homogenate of chick cerebrum | 115 | 53 | 42 |

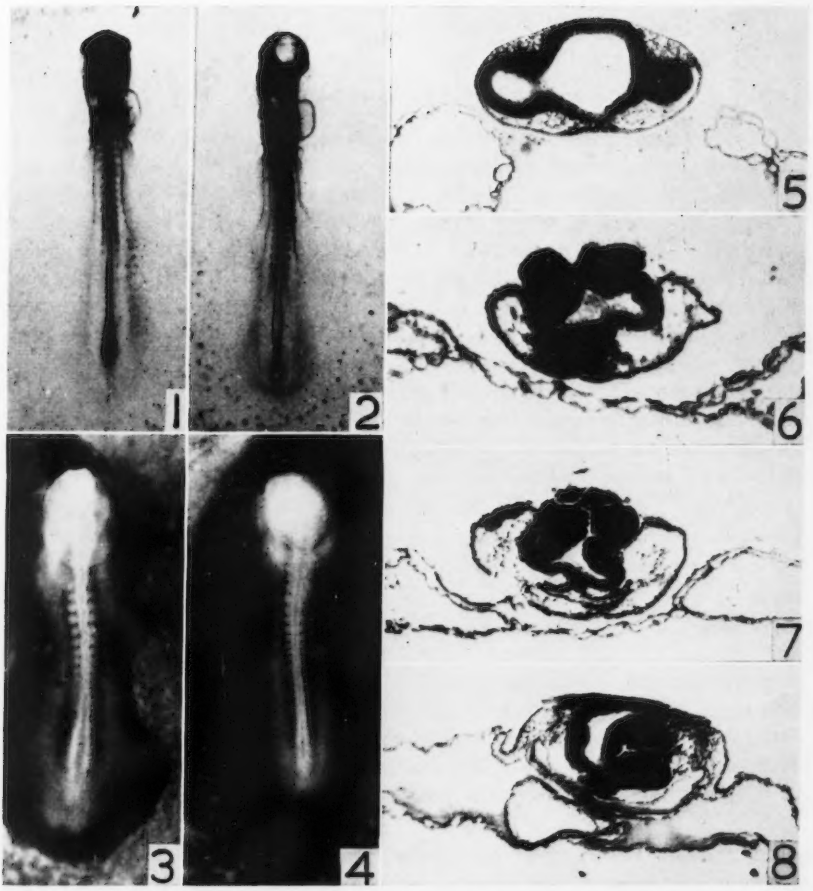


FIG. 1. A normal chick embryo of 16 somites (45–49 hours' incubation). ×12.5.
FIG. 2. A normal chick embryo of 19 somites (48–52 hours' incubation). ×12.5.
FIG. 3. An embryo from an egg injected with homogenate of chick cerebrum. Ten somites, incubated 48 hours. ×15. Note opaque, poorly differentiated brain and absence of optic vesicles.
FIG. 4. An embryo similar to that in Fig. 3, same age, 13 somites. ×15.
FIG. 5. Transverse section through the optic region of a normal 14-somite chick embryo. H and E. ×45.
FIGS. 6 and 7. Transverse sections through the brain of the embryo in Fig. 3. H and E. ×55. Note poorly closed neural tube, thick walls of brain, absence of normal optic vesicles.
FIG. 8. Transverse section through the brain of the embryo in Fig. 4. H and E. ×55. Note small ventricle and thick walls of the brain.

forebrain homogenate was significantly greater than in those treated with Locke's solution. $\chi^2$ was found to be 38.45. For $P = 0.001$, $\chi^2 = 10.83$. Hence the difference between the two groups is highly significant.

## Discussion and Conclusions

The hypothesis that differentiation proceeds as more rapidly developing regions suppress like development in more slowly developing adjacent areas, and the more slowly developing regions are thus channelled into other kinds of differentiation has been advanced by Rose (5). He has tested this hypothesis in *Rana pipiens* (6) and in *Tubularia* (7). In these experiments he has demonstrated that already well-differentiated tissues inhibit like differentiation in embryonic development or in regeneration. Lender (3) has obtained a similar inhibition in the regeneration of *Planaria* using breis of older tissues. The authors (1) have found that cell-free homogenates of adult frog brain suppress differentiation of the brain in developing frog embryos. The use of the chick embryo in the present investigation has provided a more critical test of Rose's hypothesis than the use of the frog embryo. The results are more clear-cut and more easily classified. Thus, the results could be more readily tabulated and subjected to statistical analysis.

Statistical analysis of the results shows quite clearly that the incidence of specific brain abnormalities in embryos exposed to brain homogenates is very significantly greater than in control embryos. The occurrence of brain abnormalities in embryos exposed to homogenates of other tissues was no more frequent than in untreated control embryos.

The occurrence of abnormalities in treated embryos is not related to the method of treatment. The injection of sterile Locke's solution had no effect upon the development of the embryos. Landauer (2) has used a similar technique in a different kind of investigation. He found no leakage of material out of the yolk and no apparent increase in the numbers of developmental abnormalities.

It is reasonably certain from our experiments, both in the frog and in the chick, that cell-free homogenates of well-differentiated tissues contain substances which suppress normal differentiation and development of homologous tissues in the embryo. The evidence indicates that the inhibitory substances are thermolabile. Our study in this respect is not sufficiently extensive and further investigation is required. Some work in progress and soon to be published by Lenicque (3) should elucidate the biochemical nature of the inhibiting substances.

Homogenates of mouse brain had no inhibiting effect upon the development of the brain of the chick embryo. This conflicts with the observation that homogenates of chick brain suppressed the development of the brain in the frog embryo (1). Too few experiments have been made to allow any definite conclusions to be drawn. Further investigation is necessary to determine whether the inhibitory substances are species-specific.

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# BLACK FLIES (DIPTERA: SIMULIIDAE) OF THE FORESTS OF QUEBEC[1]

L. S. WOLFE[2] AND D. G. PETERSON[3]

## Abstract

Twenty-two species of black flies, three of which are new to science, were found in the Baie Comeau area of Quebec in 1954 and 1955. *Simulium venustum* Say constituted 75 to 80% of the population and was the major species biting man. *Prosimulium hirtipes* (Fries), *S. parnassum* Mall., and *S. tuberosum* (Lund.) were also pest species. *S. verecundum* S. & J. was identified in collections made in August, 1955. Accounts are given of the habits, dispersal, transportation, stream-current preferences, feeding, and predators of the larvae. The structure of the unique pupal cocoon of *Cnephia invenusta* (Wlk.), and the formation of its stalk from the stems of dead moss, were studied.

## Introduction

A study of the biology and control of biting flies in the forests of Eastern Canada was initiated in 1954. The objectives of the program were to identify the species, to ascertain their life histories and habits, and to evaluate and develop control and protective measures.

The studies on biology were conducted on the north shore of the St. Lawrence River near Baie Comeau, Quebec. In 1954, the field party was stationed at Manicouagan Depot, near Baie Comeau, and most of the collections and observations were made in the George Tremblay River and English River drainage basins. In 1955, a field station, including a meteorological station and an insectary, was established at the mouth of Brisson Creek, on the English River, approximately 25 miles north of Baie Comeau (Fig. 1). Black flies infested numerous streams within a few miles of the field station.

The species recorded at Baie Comeau and general aspects of their life histories and habits are described in this paper.

## Weather Conditions

The most striking difference between the weather conditions of the summers of 1954 and 1955 in the Baie Comeau area was in the amount of rainfall (Fig. 2). The 1955 season (from May 20 to August 25) was the driest (6.3 in. of rainfall) since records were commenced in 1944, whereas the 1954 season was the wettest (17.8 in.).

Wind velocities recorded at the field station in 1955 were remarkably light, the maximum recorded being 15 m.p.h. On an average day, the wind speed

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[2]Veterinary and Medical Entomology Unit, Ottawa.
[3]Veterinary and Medical Entomology Unit, Ottawa; now at Entomology Laboratory, Guelph, Ontario.

rose from 2 to 4 m.p.h. at 7 a.m. to 9 to 12 m.p.h. at 1 p.m. and then decreased to 2 to 4 m.p.h. after sunset. At midnight it was generally calm. Relative humidity records showed a uniform daily variation from 40 to 50% at noon to 95 to 100% during most of the night.

The ice broke up in early May in both 1954 and 1955, although large lakes were not free of ice until early June. In 1955, temperatures of waterways were above 32° F for approximately 6 months and varied between 50° and 65° F from June to August. Maximum stream temperatures of over 70° F were recorded occasionally during the hot weather in July of that year.

## Topography

The terrain is rugged with narrow, deep valleys separated by smooth ridges with steep, rocky slopes typical of the Canadian shield. The soil is shallow and the drain-off is rapid, causing water levels to increase greatly a

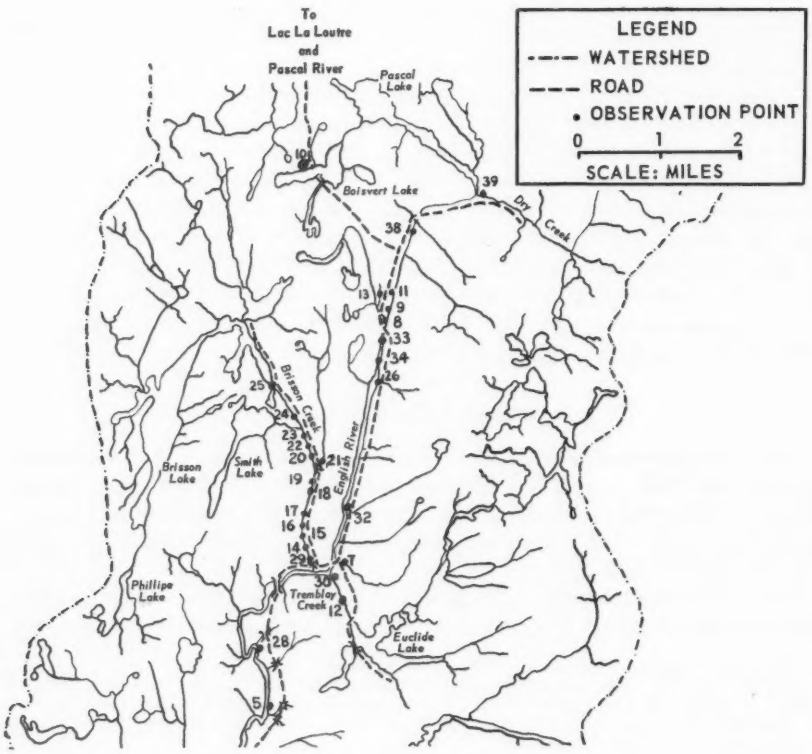


FIG. 1. Northern section of the English River drainage basin near Baie Comeau, Quebec, showing sites of observations in streams.

short time after rain. The area has typical hydrographic and topographic conditions for black-fly country as defined by Hocking and Richards (22), with many lakes, fast-flowing streams and creeks, rillets, seepages, torrents, waterfalls, and large rivers (Fig. 1).

The forest is a mixture of spruce, balsam, jack pine, alder, poplar, larch, and birch. The birch has been ravaged by dieback.

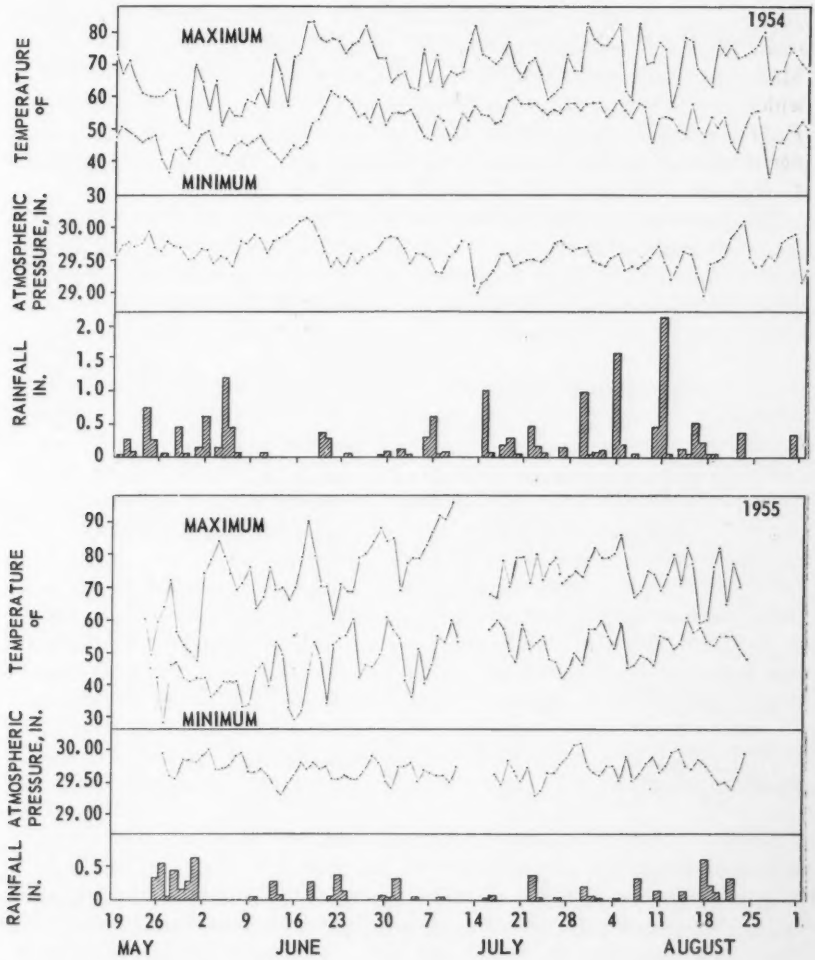


FIG. 2. Weather records, Baie Comeau, Quebec, 1954 and 1955. The 1954 data were recorded at Manicouagan Depot; those for 1955 at the field station in the English River drainage basin until June 11, when, because of forest fires, the equipment was relocated at the Depot.

## Species Present

The 22 species recorded in the Baie Comeau area are listed in Table I. Twenty-four species have been reported from Goose Bay, Labrador (22), of which the following were not found at Baie Comeau: *Prosimulium* (*Prosimulium*) *ursinum* Edw., *Simulium* (*Eusimulium*) *croxtoni* N. & M., *Gymnopais holopticus* St., and *Twinnia tibblesi* S. & J. The following species recorded in Ontario (8, 10, 39, 43) were not found at Baie Comeau: *P.* (*Helodon*) *gibsoni* Tw., *P.* (*H.*) *vernale* Shew., *P.* (*H.*) *decemarticulatum* Tw., *S.* (*E.*) *rivuli* Tw., *S.* (*E.*) *innocens* Shew., *S.* (*S.*) *fibrinflatum* Tw., and *S.* (*S.*) *jenningsi* Mall. In upper New York State, 23 species have been collected (42), of which the following were not found in the Baie Comeau area: *T. tibblesi*, *P.* (*P.*) *rhizophorum* S. & J., *C.* (*E.*) *loisae* S. & J., *S.* (*E.*) *gouldingi* St., *S. fibrinflatum*, *S. jenningsi*, and *S. pictipes* Hgn.; *C. loisae* is closely related to *C. invenusta*, which was recorded at Baie Comeau.

The following information was obtained from observations in many localities in the Baie Comeau area from May until September, 1955, unless otherwise stated. Most of the streams were examined at 2-day intervals and two (Tremblay Creek and Brisson Creek) were examined several times each day.

TABLE I

The species of black flies recorded in Baie Comeau area, Quebec, 1954 and 1955

*Cnephia* End.
  *Cnephia* End.
    *dacotensis* (D. & S.)
  *Ectemnia* End.
    *invenusta* (Wlk.)
  *Mallochianella* V. & D.
    *mutata* (Mall.)
  Subgenus ?
    sp. L
*Schoenbaueria* End.
  furculata (Shew.)
*Simulium* Latr.
  *Aspathia* End.
    *parnassum* Mall.
  *Byssodon* End.
    *rugglesi* N. & M.
  *Eusimulium* Roub.
    *aureum* Fries
    *latipes* (Meig.)
    *pugetense* D. & S.
  *Gnus* Rubtz.
    *corbis* Tw.
  *Hellichia* End.
    *euryadminiculum* Dav.
    sp. near *subexcisum* Edw.
  *Neosimulium* Rubtz.
    *vittatum* Zett.
  *Simulium* Latr.
    *decorum* Wlk.
    *tuberosum* Lund.
    *venustum* Say
    *verecundum* S. & J.
  Subgenus ?
    sp. M, near *pictipes* Hgn.
*Prosimulium* Roub.
  *Prosimulium* Roub.
    *hirtipes* Fries
    *multidentatum* Tw.
  *Helodon* End.
    *pleurale* Mall.

### *Cnephia dacotensis*

*C. dacotensis*, a nonbiting black fly, has been reported to be a spring species with a restricted habitat and to deposit eggs that remain in diapause until the following spring (8, 21, 22, 31, 42). This was confirmed in the Baie Comeau area. Small colonies of pupae were found from June 7 to 20, associated with *S. decorum* and *S. venustum*, at the outlet stream from Lake Louise on the Wood River system. Pupae were also found in the George Tremblay River. Male and female adults were captured on June 20 and 22, crawling

and flying over stones and moss at the outlet of Lake Louise. The females were newly emerged and gravid. Nicholson (30) and Krafchick (27) reported that the eggs mature during the pupal stage and copulation takes place immediately after emergence.

Downes (12) suggested that the males of this species do not form organized mating swarms and related this to the eye structure. The eyes are not clearly divided into regions of large and small ommatidia as is usual in other species.

The species has been recorded in Canada from northern Manitoba, Ontario, Quebec, and Labrador (8, 21, 22, 43).

*Cnephia invenusta*

This species overwintered as a larva and was the first black fly to emerge in the Baie Comeau area. Mature larvae and newly formed pupae and cocoons were obtained in mid-May when collecting commenced. The pupae were invariably found at a depth of 3 to 4 ft in swiftly flowing, unbroken waters of permanent streams of the adolescent type. At most, three pupae were found attached to each stone but usually only one. Only empty cocoons were found after May 29, when the water temperature had risen to 45° F. Collections indicated a wide distribution (e.g., Brisson Creek, English River, Papinachois Falls).

Larvae were found attached to trailing moss, *Hygrohypnum* sp., in the stream. When mature, the larva formed a stalk composed of macerated stems of dead moss and detritus, ensheathed by a dense layer of hardened silklike fibers. The black stalk, 10 to 15 mm long, was fixed to a stone by a circular holdfast formed of many strands of a silklike material. The distinctive pocket-shaped cocoon (Fig. 3) was spun on the under side of the attachment stalk. It is 5 to 7 mm long and 1 to 2 mm broad at the mouth, and elliptical in cross section. The cocoon is formed from loosely woven threads with a single terminal aperture of irregular margin. The pupa lies with its ventral surface along the stalk, the anterodorsal part of the thorax filling the cocoon aperture. The two respiratory organs are each composed of eight fingerlike filaments, 1.5 mm thick, that are covered by a pattern of small globular thickenings of their walls.

Numerous males and females were reared from pupae. *C. invenusta* is not attracted to man and only one female was netted—this was at Brisson Creek, on June 20.

Apart from the Baie Comeau region, the species has been collected in Canada only at Laniel, Quebec, and the type locality at St. Martin's Falls, Albany River, Hudson's Bay. It is closely related to *C. taeniatifrons*, recorded in Western Canada, and *C. loisae*, in the Adirondacks.

*Cnephia mutata*

*C. mutata* had one generation a year and overwintered in the larval stage. The larvae were easily recognized by their distinctive brown color. They were usually found on small stones and pebbles in shallow waters of forest streams, seepages, and small permanent streams of the infant and young types.

Although associated with *P. hirtipes*, the larvae of *C. mutata* were most often found in small runoffs with a swift but not turbulent flow. Pupation occurred from May 23 to the end of June and adults were netted from early June until August. *C. mutata* was not noted to be attracted to or feed on man. Male and female adults were usually collected in sweeps over border vegetation.

*C. mutata* has been recorded in Manitoba, Ontario, Quebec, and Labrador (8, 21, 22, 39, 43).

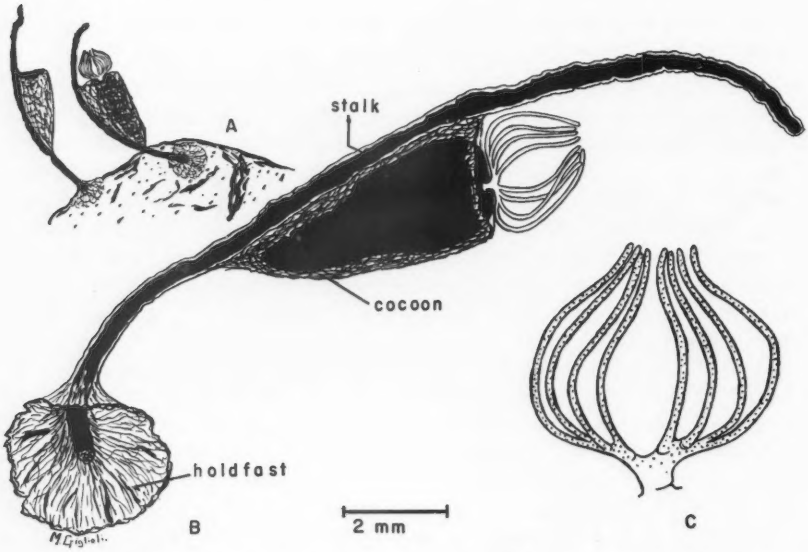


FIG. 3. Pupa and cocoon of *Cnephia invenusta* Walker: A, manner of attachment to rocks; B, structure of pupal cocoon; C, arrangement of pupal respiratory filaments.

*Cnephia* sp. L.

Four female adults of an undescribed species were collected near Lac la Loutre on July 10, along the English River on July 18, and on the Manicouagan Peninsula on July 30.

*Schoenbaueria furculata*

Two females were collected on the upper Antrim River on July 6, 1954. It is typically an early species found in considerable numbers in the Yukon Territory, Northwest Territories, northern Manitoba, and Quebec (21, 39). A few specimens have been collected in southern Canada.

*Simulium parnassum*

Twenty-two females were collected at Brisson Creek and Moose Creek between mid-July and the end of August. The eggs, larvae, pupae, and male adults were not found, and nothing is known of the life history. Specimens were captured while biting humans but, because of the rarity of the species,

it is not an important pest. Apart from the Baie Comeau region, this species has been reported in Canada only from Goose Bay (22) and Algonquin Park, Ontario (8). It has been recorded in New Hampshire, New York, Virginia, and Maryland (45).

*Simulium rugglesi*

Adults of this species were collected from July 21 to August 8. It is a midsummer species and probably has only one generation a year. It has been recorded in Labrador, Quebec, New Brunswick, and Ontario (40), but is more common in the Prairie Provinces, where it is a pest of domestic and game birds and transmits the blood parasite *Leucocytozoon simondi* (16).

*Simulium aureum*

This is a rare species in the Baie Comeau area. Pupae were found in Brisson Creek, on June 25, and in the Outardes River on July 27, suggesting that there are two generations each year, as in other localities (8, 13, 17, 24, 32, 42, 43). Thirty-five females were collected between mid-July and late August in sweeps near observers in the forest. It was not observed biting. The species has an extensive distribution throughout Europe and North and Central America, and has been collected from all Canadian provinces (8, 18, 21, 22, 43). The immature stages have been extensively studied (11, 28, 33, 42, 43).

*Simulium latipes*

Of the species that overwintered as larvae, *S. latipes* was the last to emerge. Mature larvae were found associated with *C. mutata* in cold (40° F) forest streams in late May and were followed through to emergence. There were two generations each year. This species similarly overwinters in the larval stage in Britain (13, 14). Stone and Jamnback (42) reason that in the Adirondacks it overwinters in the egg stage since no larvae are found until May.

*S. latipes* is annoying to man in Britain and has been observed biting (13, 14). In Canada, it makes no attempt to feed on man (8), although in the Baie Comeau area it was frequently collected in swarms around observers.

*S. latipes* has been recorded in the spring and early summer in the Northwest Territories, northern Manitoba, Ontario, Quebec, and Labrador (8, 21, 22, 43).

*Simulium pugetense*

Eight pupae of this species were collected from forest streams on August 8. The pupal cases were separated from those of *S. euryadminiculum* by their smaller size, more nearly flat and broader shape, and the greater thickness of the dorsal pair of respiratory filaments. Although Stone and Jamnback (42) state that the adults of this species cannot be separated from those of *S. latipes*, males were readily separated by means of Twinn's (43) figures of the male genitalia (figured as *quebecense*).

*S. pugetense* probably has two generations a year as in Ontario (8). It has a wide Canadian distribution (8, 18, 22, 43).

*Simulium corbis*

The larvae of this species were found firmly attached to rock faces and stones within and immediately below cascades and waterfalls in which the temperature was from 56° to 59° F. The pupae, although occurring in the rapids below waterfalls, were more concentrated in the turbulent backwaters beneath the waterfalls. Only one generation was found. Male and female adults were netted from border vegetation and over water immediately above waterfalls. Oviposition was also noted at these sites, the eggs being deposited singly by the females while in flight over the rapids above the falls.

Females were taken in small numbers in sweeps near observers but the species was not a pest. Hocking and Richards (22) noted that it was attracted to and probably bites man in Labrador.

The species is found throughout Canada (18, 22, 43).

*Simulium euryadminiculum*

This species overwintered in the larval stage, and pupation occurred from May 25 to June 10, while the stream temperature rose from 45° to 55° F. A heavy infestation of the immature stages, associated with those of *S. vittatum*, *S. latipes*, and *P. hirtipes*, was observed on stones in a clear, shallow rill below the dam in Brisson Creek. There was a single generation each year, and a summer diapause in the egg stage. The females did not attack man and the adults were collected only by sweeping over the vegetation bordering the stream.

The type locality is in Ontario (7). Specimens have also been collected at Baker Lake, N.W.T., in southern and northern Quebec, and in Labrador (7, 22).

*Simulium* sp. near *subexcisum* Edw.

Six females of this rare but widely distributed species were taken at Station No. 24, Brisson Creek, on June 23, and in the forest on Manicouagan Peninsula on July 30. It probably has one generation a year.

The specimens collected at Baie Comeau fit the description of *S. subexcisum* given by Twinn (43), but are nevertheless certainly distinguished from *Eusimulium subexcisum* (Edw., 1915).

*Simulium vittatum*

This was one of the commonest species throughout the summer, and had a general distribution. Three large generations and a small fourth one were observed. Four generations have also been reported in Alaska (25), Saskatchewan (19), Illinois (17), and South Carolina (26). In contrast, a single generation has been reported in Labrador (22), and two in Algonquin Park, Ontario (8).

*S. vittatum* overwintered in both the egg and the larval stages. In the first generation, comparatively few of the pupae developed from overwintered larvae. A heavy second wave of pupation, from overwintered eggs, occurred concurrently with the pupation of the first generation of *S. venustum*. The

distinctive strings of eggs were found on submerged birch logs on May 27, and subsequently hatched in the laboratory. It is inconceivable that these eggs had been deposited previously in that season by either local or immigrant black flies, since the development time from egg to adult, in the streams, was approximately 30 days. Overwintering larvae have been recorded previously (3, 18, 43, 44), but not overwintering eggs. In the late summer, when many stones were covered with algae, the larvae were found almost exclusively on aquatic bur reeds, eelgrasses, hornworts, and water milfoil (*Sparganium* sp., *Valisneria americana*, *Zannichellia palustris*, *Ceratophyllum* sp., and *Myriophyllum spicatum*).

Emery (15) and Wu (46) have described the eggs and immature stages of *S. vittatum*, the latter author stating that the eggs were laid 5 days after emergence. Observations in the Baie Comeau area indicated that oviposition by females of the first generation could occur within 24 hours of emergence, and that a blood meal was not always required by this species for maturation of eggs of the first ovarian cycle. Males emerged before females and a mating swarm was observed on the evening of the day that the females emerged. Next day, on June 5, freshly deposited eggs and gravid females were found. Dissection of the females showed no signs of a recent blood meal. We presume that subsequent oviposition, which was observed periodically through the season, always required a blood meal.

*S. vittatum* was not observed to bite man, although it was present in considerable numbers in the black-fly cloud around man. Davies (8) has likewise reported that *S. vittatum* does not attack humans; however, in Western Canada, it does bite man, as well as cattle, horses, and moose (3, 19).

Third and fourth generation adults were much smaller (1.5 to 2.5 mm in length) than those of earlier generations. Rubtzov (38) also reported this for certain Russian black flies and related it to the higher stream temperatures in late summer.

Adults were collected as much as 10 miles from the nearest breeding areas. The species is found throughout North America (8, 18, 21, 22, 43).

*Simulium decorum*

This common species had two generations a year and overwintered in the egg stage, as was also found in Ontario (8). The first generation was the largest. The larvae and pupae were found in large numbers to a depth of 2.5 ft on the lakeside faces of sluice-gate boards of dams at the outlets of lakes. Pupae were also found at most lake outlets but not in such great numbers as on dams. The presence of numerous dams in logging areas has probably led to an increase in the abundance of this species.

The first males emerged 2 to 3 days before the females, and remained on the shaded parts of the dam structure. Mating occurred within 24 hours of emergence of the females. The females were gravid on emergence and, without a blood meal, oviposited within 48 hours. They laid eggs singly and in groups on the dampened logs in the spray zone of the same dam from which they had emerged. The eggs hatched in 6 to 10 days and the small

larvae moved against the current along the boards of the dam onto boards over which the full current of the sluiceway flowed. Mature female pupae had a bulky fat body which may have sufficed for the development of the eggs without a blood meal and enabled oviposition to occur immediately after copulation.

*S. decorum* was attracted to man but did not bite. It is widely distributed throughout Canada (8, 18, 21, 22, 43).

*Simulium tuberosum*

Four generations a year were found in the Baie Comeau area, as in the Adirondacks (42). The third generation was by far the largest. The larvae, which can be separated from those of *S. venustum*, were found in the spring, summer, and early autumn associated with those of *S. venustum* in all types of permanent streams and rivers.

Oviposition of this species was noted for the first time on record in late August, 1956, at the outlet of Pope Lake. A group of six females were observed laying eggs in the calm water on the shore of the lake among the stones just above the lake outlet. The flies alighted on the water surface and each immediately poured out a stream of 10 to 20 eggs, which sank slowly to the lake bed among the rocks. The flies then rose from the water surface, flew around close to it for a short time, and repeated the process in a new location. The females did not deposit eggs while in flight. The eggs, similar in size and shape to those of *S. venustum*, were separate and not held together in a string by a gelatinous envelope. Rubtzov (38) stated that, in Russia, *S. tuberosum* lays eggs in clusters like those of *S. venustum*. Davies and Peterson (10) did not find this in Algonquin Park, Ontario, and suggested that this species oviposited while flying.

Although Stone and Jamnback (42) did not distinguish the females of *S. venustum* from those of *S. tuberosum*, we found that the two species are readily separable. *S. tuberosum* is smaller and darker, has dull-gray anterior tibiae with the silvery area not covering more than two-thirds of their width, has a brown or black pleural tuft, and brown to black hairs at the base of the costa and on the stem vein.

The species has been reported attacking man, as well as horses and other domestic animals (10, 43). It did not usually bite man at Baie Comeau but was attracted to him and was one of the annoying species in the black-fly cloud. In August, *S. tuberosum* was captured biting man at only one locality. All flies emerging from a nearby creek at this time were of *S. tuberosum*.

The species is Holarctic in distribution and has been recorded from all Canadian provinces and from Alaska (8, 18, 21, 22, 43).

*Simulium venustum*

This was the most abundant species throughout the summer, constituting 75 to 80% of the black-fly population, and the most important species biting man. Development of the larvae, pupation, emergence, and oviposition were followed through three generations and possibly a fourth, by daily

observations at a number of localities. Examination of the male terminalia (42) showed that *S. venustum* was not being confused with *S. verecundum* in the first and second generations, but that it possibly was in the third and fourth as males of *S. verecundum* were first determined in a collection made on August 18. The interrelation of these two species in August and possibly late July requires careful reinvestigation.

Twinn (43) stated that this species overwinters in the larval stage, whereas Stone and Jamnback (42) consider that it overwinters in the egg stage. From May 16 to 20, immediately after the ice breakup in the Baie Comeau area, most of the larvae in the streams were the mature larvae of *P. hirtipes*. Many minute larvae of *S. venustum* appeared after May 23, and these were assumed to develop from overwintered eggs. The species may also overwinter in the larval stage. A few small larvae had been noted with those of *P. hirtipes*, and it is possible that our observations were not extensive enough to exclude the presence, at that time, of large numbers of the minute, overwintered first-instar larvae of *S. venustum*.

The observation of three generations is at variance with Stone and Jamnback's (42) statement that this species apparently has only one generation a year. The first and second generations at Baie Comeau were the largest and the third generation was small in numbers, though multivoltine species of black flies, like *S. tuberosum* and *S. vittatum*, usually have a small first generation and the population builds up gradually during the summer. Fredeen (19) has also observed that *S. venustum* becomes less abundant with each succeeding generation, unlike *S. vittatum* and *S. tuberosum*.

*S. venustum* oviposits on stones and logs at the water line if floating vegetation is not available. This was the situation in the spring, whereas in the summer and autumn the eggs were found on the upper surfaces and borders of trailing vegetation in the calmer waters immediately above outlets from lakes or the calmer reaches of streams. The floating vegetation, with the egg masses attached, dies with the approach of winter and is carried to the stream bed.

Larvae and pupae were found in a wide variety of stream types, ranging from torrents to slow-moving creeks and ditches. They were never found in deep waters. The heaviest infestations were found in rill sections and small rapids at the outlets of lakes and pools. In July and August, the larvae were more abundant on aquatic grasses than on stones, since most of the stones were covered with black and brown algae at this time of the year.

The enormous number of eggs laid by first generation adults of *S. venustum* did not produce a corresponding number of second generation larvae. Masses of eggs were found attached to almost every available floating blade of grass above rill sections of Brisson Creek. Microscopic examination of these egg masses at various times during the season showed that only about 50% of them had hatched. An explanation of these findings will depend on further study. Three possibilities are: (1) two types of eggs are deposited by first-generation females, one type hatching immediately and providing second-generation larvae, and the other type entering diapause until the following

spring; (2) many of the eggs are not viable; and (3) two species or subspecies of black flies are present which, although taxonomically inseparable, possess different habits.

Six gynandromorphs of *S. venustum* were found in a sweep collection of 12 oz, or approximately 300,000 black flies. In three of the specimens, the thorax and abdomen were divided longitudinally and the head was either male or female. Three other specimens were divided transversely with a male thorax, abdomen, and hind legs but with a female head. Sexual mosaics have been reported for black flies by Puri (33).

Several black flies were collected that agreed with *S. venustum* in all characters except that the dorsum of the thorax was exceptionally hump-backed, a characteristic of the females of *S. decorum*.

*Simulium verecundum*

*S. verecundum* was first described by Stone and Jamnback (42) from New York, and named on the basis of its nonannoying habits. It appears to be widespread in North America. Six males of this species were found among prepared terminalia of males collected at Baie Comeau and previously thought to be of *S. venustum*. All the specimens were reared from pupae collected from below the main dam of the Manicouagan River on August 18. This was the last collection of males made in the Baie Comeau area.

Little can be said about the biology of this species at present except that it appears to be a late-summer species and to occur along with *S. venustum*. The larva and pupa are not separable from those of *S. venustum*. There are also no reliable characters in the females to distinguish them from *S. venustum*, so that, at present, identification of this species depends entirely on collections of males.

The large series of *S. venustum* females collected during the summer showed intergradation of the size and color of the pleural tuft and stem vein hairs. Some specimens collected in August had ovipositor plates that were more incurved medially and were separable from specimens collected in June and July, which had the ovipositor plates straight and parallel medially. Whether the former specimens are referable to *S. verecundum* is not known. No other distinctive differences in prepared genitalia of spring and autumn specimens were found.

*Simulium* sp. M near *pictipes* Hgn.

Large numbers of larvae and pupae of this species were found at Outardes Falls on July 18, attached to rocks over which water was flowing swiftly. The immature stages were restricted to this type of environment, which corresponds to that described for *S. pictipes*. Eggs were found in a single layer in the spray zone and on rocks intermittently flooded by the surge of water over the falls. Two generations a year were found.

Males were captured in large numbers in swarms over the falls, whereas females were netted several miles away, feeding on the nectar of blueberry flowers. This species did not bite man but was frequently netted in sweeps

near observers. It was first thought that this species was *S. pictipes*; however, the male and female genitalia and the shape of the pupal respiratory filaments are different from those of that species.

*Prosimulium hirtipes*

*P. hirtipes* was the common black fly in early June and the major species biting man in the early spring. It had one generation a year and overwintered in the larval stage.

Oviposition was observed at Tremblay Creek during the afternoons of June 1 and 2, the first and second days after the first females emerged. The females hovered 6 to 12 in. above the swiftly flowing water, close to the banks, and frequently darted to the water surface, touched it, paused 1 to 3 seconds, then rose, hovered a moment, and repeated the act. The eggs were laid singly or in batches of three to four held together by a secretion. Females captured while behaving in this manner were gravid.

The eggs hatch in late autumn and the larvae probably develop slowly during the winter months. Only large larvae of *P. hirtipes* were found when Brisson Creek was first examined on May 18, when the water temperature was 41° F. Small streams that had dried out during the summer were heavily infested with larvae of *P. hirtipes* the following spring; however, even though no water flowed in some watercourses in August (e.g., Tremblay Creek), the substratum beneath the stones was always moist. The species most commonly associated with *P. hirtipes* in streams were *C. mutata*, *Schoenbaueria furculata*, and *Simulium latipes*.

Immature stages were found in watercourses in May, June, and early July. In the cooler waters of small, spring-fed streams, development was retarded. Table II indicates the effect of stream type and water temperature on the time of pupation and emergence. The heaviest infestations of larvae of *P. hirtipes* were found in small, shallow waterways with many cascades and rapids and a rock bed (e.g., Tremblay Creek). This stream type corresponds to the "young stream" in Dalmat's (5) classification of watercourses.

TABLE II

Dates of first pupation and emergence, and levels of infestation of *Prosimulium hirtipes* (Fries) in various watercourses in Baie Comeau area, Quebec, 1955

| Watercourse | First pupation | | First emergence | Level of infestation |
|---|---|---|---|---|
| | Date | Water temp.,* °F | | |
| Tremblay Creek | May 20 | 43 | May 28 | Very heavy |
| Brisson Creek | May 22 | 44 | May 30 | Moderate |
| English River | May 24 | 42 | May 31 | Moderate |
| Forest stream | | | | |
| Station No. 10 | June 1 | 44 | June 8 | Moderate |
| Station No. 13 | June 3 | 43 | June 11 | Light |
| Spring-fed stream | | | | |
| Station No. 32 | June 11 | 45 | June 22 | Light |
| Station No. 7 | June 16 | 42 | June 28 | Very light |

*Mean daily temperatures measured by a recording thermometer in Brisson Creek, maximum—minimum thermometers in Tremblay and Brisson Creeks, and by mercury thermometers in other streams.

The dates recorded for emergence of *P. hirtipes* in the Goose Bay region (22) were 1 month later than those for the Baie Comeau region, whereas those for the Adirondacks (42) were approximately 1 month earlier. The dates of emergence in the Ottawa district (43) fall between those at Baie Comeau and the Adirondacks.

Adults were observed emerging from small cascades and waterfalls. After emerging from the pupal stage, the adults rose to the surface surrounded by many tiny air bubbles clinging to the hydrophobic cuticle, and were projected into the air by the turbulence of the current. They immediately flew to the border vegetation, where they alighted and rested on the under surface of the needles and branches of balsam firs and white spruce. Males commenced emerging 2 to 3 days before females. Females were not taken biting man before June 7, which was 1 week after the peak of emergence and also after oviposition was first observed. Hence, we conclude that *P. hirtipes* evidently does not always require a blood meal for the first ovarian cycle. Stone and Jamnback (42) also recorded that adults do not bite for at least a week after emergence.

*P. hirtipes* has been already subdivided into several groups on cytological grounds (34, 35). Pupae with 14, 15, and 17 respiratory filaments were collected in the Baie Comeau area together with the usual 16-filamented type. Rearing showed that the 14-filamented pupae were not mistaken for those of *P. ursinum*. The female adults varied considerably in color. The body was dark brown but the legs and hairs varied from light yellow through orange to black-brown. The very dark specimens were difficult to separate from the females of *P. multidentatum* and were collected more frequently in July and August.

*Prosimulium multidentatum*

One pupa and several empty pupal cases and cocoons of this species were collected on June 15, 1955, at a depth of 2 to 3 ft in swiftly flowing waters above the falls of the Outardes River. No adult males were collected. *P. multidentatum* is evidently a spring species as reported from Goose Bay and northern Manitoba (22), as well as from the area of Ottawa, Ontario (43).

*Prosimulium pleurale*

The collection of two males of this species on June 16 and 23, 1954, is the only record for Eastern Canada. The species is more common in northern British Columbia, Yukon Territory, Northwest Territories, and Alaska.

## General Observations

The chronological succession of black-fly generations in the Baie Comeau area in 1955 is summarized in Table III. At the beginning of the season, each larval and pupal population was clearly defined and the overwintered eggs and larvae resulted in two, clearly-defined groups within the first generation. The heaviest infestation of streams was from June 5 to 20 and was

derived from overwintered eggs. There was considerable overlap between the larval and pupal populations in July and August. The effect of immigrant black flies, as suggested by Davies (8), was not observed.

TABLE III

Dates of first appearance of black flies by generations in Baie Comeau area, Quebec, 1955

| Species | First adults |
|---|---|
| First generation | |
| From overwintered larvae | |
| *C. invenusta* | May 27 |
| *P. hirtipes* | May 28 |
| *S. vittatum* | June 3 |
| *S. euryadminiculum* | June 5 |
| *C. mutata* | June 7 |
| *S. latipes* | June 17 |
| *P. pleurale* | * |
| *P. multidentatum* | * |
| From overwintered eggs | |
| *S. venustum* | June 3 |
| *S. tuberosum* | June 10 |
| *S. vittatum* | June 12 |
| *S. decorum* | June 14 |
| *S. corbis* | June 17 |
| *C. dacotensis* | June 20 |
| *S.* sp. near *subexcisum* Edw. | June 23 |
| *C.* sp. L | July 10 |
| *S. parnassum* | July 14 |
| *S. aureum* | July 15 |
| *S.* sp. M | July 17 |
| *S. rugglesi* | July 21 |
| *S. furculata* | * |
| *S. pugetense* | Late July |
| *S. verecundum* | August 18 |
| Second generation | |
| *S. venustum* | June 26 |
| *S. vittatum* | July 5 |
| *S. tuberosum* | Early July |
| *S. decorum* | Late July |
| *S. latipes* | * |
| *S. pugetense* | * |
| *S. parnassum* | * |
| *S. aureum* | Late August |
| *S.* sp. M | August |
| Third generation | |
| *S. venustum* | July 25 |
| *S. vittatum* | August 1 |
| *S. tuberosum* | August 5 |
| Fourth generation | |
| *S. venustum*† | Sept. |
| *S. vittatum* | Sept. |
| *S. tuberosum* | Sept. |

*Insufficient records.
†Possibly *S. verecundum*.

*Larval and Pupal Environments*

We have adapted Dalmat's (5) classification of stream types to relate the black-fly fauna to the stream characteristics.

1. Infant streams: temporary, seepage waters, not more than 2 ft in width, with no distinct bed other than a few bare stones. The species present were *P. hirtipes* and *C. mutata*.

2. Permanent spring-fed forest streams, 2 to 6 ft in width (e.g., stations No. 7, No. 13, and No. 32). The species present were *P. hirtipes*, *C. mutata*, *S. latipes*, *S. venustum*.

3. Young streams: permanent streams 6 to 15 ft in width, with a stony bed and banks, numerous cascades, torrents, rapids, and waterfalls, and with dense border vegetation. The species present were *P. hirtipes*, *C. mutata*, *C. dacotensis*, *S. venustum*, *S. tuberosum*.

4. Adolescent streams: small rivers, interrupted occasionally by falls, and with many rapids and rill sections, a graded stony bed, and a lake as a source (e.g., English, Wood, and Antrim Rivers and Moose Creek). The species

present were *P. hirtipes*, *C. mutata*, *C. invenusta*, *S. latipes*, *S. euryadminiculum*, *S. vittatum*, *S. venustum*, *S. tuberosum*, *S. corbis*, *S. decorum*, *S. aureum* (Fig. 5).

5. Mature and old streams: large rivers with great flow, many sluggish sections, a rocky or sandy bed, occasional large waterfalls, and little vegetation at the water's edge (e.g., Manicouagan and Outardes Rivers). The species present were *S. venustum*, *P. multidentatum*, *S. aureum*, *S. rugglesi*, *Simulium* sp. M, *S. tuberosum*.

6. Dams and sluiceways almost exclusively inhabited by *S. decorum* (Fig. 4).

*Habits and Preferences of Larvae and Pupae*

The larvae preferred to attach to surfaces over which the stream flow was laminar, as was reported previously by Grenier (20) and Hocking and Pickering (21). This was generally the situation on the upstream sides of logs and stones on the stream bed (Figs. 6 and 10). They also attached in greater numbers in those parts of the strean where the current velocity ranged from 1/3 to 1 ft/sec. *P. hirtipes*, *Simulium* sp. M, and *S. decorum*, however, were often found in waters where the current velocity was greater than 1 ft/sec. The current provides the mechanical force that moves the larvae to sites favorable for attachment (21). Tactile and visual stimuli presumably complete the process. The current, besides providing most of the energy for locomotory activity, assures an abundant supply of food and oxygen. In relating current properties to attachment, two entities must be considered: the macrocurrent which delimits the sections of a stream that are suitable for attachment; and, the microcurrent that determines the suitability of a particular surface on an individual rock, log, or blade of grass. The larval environment should be classified by measurements of the microcurrent rather than the rate of flow of the whole stream; unfortunately, available flowmeters are inadequate for measuring microcurrents.

Observations on the attachment of larvae to white plates and cones, on which the larvae attached readily, showed that a diurnal as well as a seasonal migration of larvae occurred, as first described by Rubtzov (36, 37, 38). In the daytime, larvae congregated in masses and during the night moved downstream. There was, therefore, a general seasonal tendency for movement of larvae from the source of the stream, where oviposition most frequently occurs, to the issue. Under normal hydrographic conditions, the larvae were not transported across lakes or calm reaches of rivers more than 100 yards long. When the creeks were in flood, as during the logging drive, the water levels were suddenly raised two or more feet, and the larvae were flushed through these sections of the streams.

Pupae usually occurred in protected situations out of the full force of the current provided there was a constant change of water reaching the pupal respiratory filaments; consequently, pupae were usually found on the downstream sides of logs and stones (Fig. 10). There is an interesting correlation, first indicated by Rubtzov (38), between the number of pupal respiratory filaments and the character of the stream. *S. euryadminiculum* has four long respiratory filaments in each tuft, and a finely woven flattened cocoon.

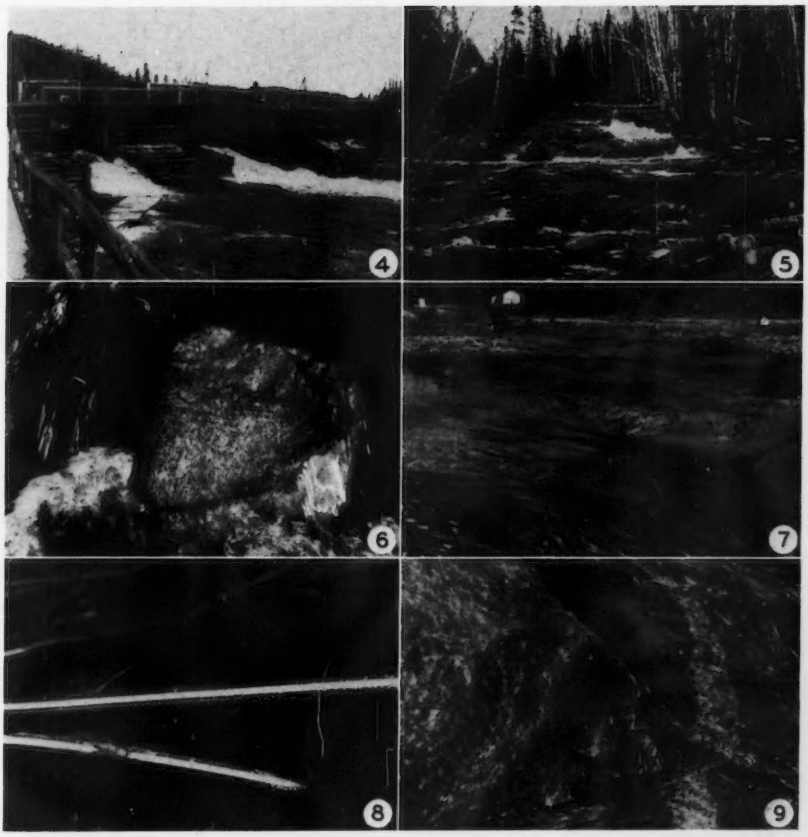


FIG. 4. The wooden dam at the outlet of Lac la Loutre, English River.
FIG. 5. The Antrim River during the logging drive.
FIG. 6. Larvae of *S. venustum* attached to a rock.
FIG. 7. Black-fly oviposition site below the dam at the outlet of Lac la Loutre, English River.
FIG. 8. *S. venustum* egg-masses on floating blades of grass.
FIG. 9. *Simulium* M egg masses on rock in Outardes Falls.

It frequently pupates in swift water. *P. hirtipes*, with 16 filaments in each tuft, and a loosely woven cocoon, invariably pupates in protected situations away from the full force of the stream flow.

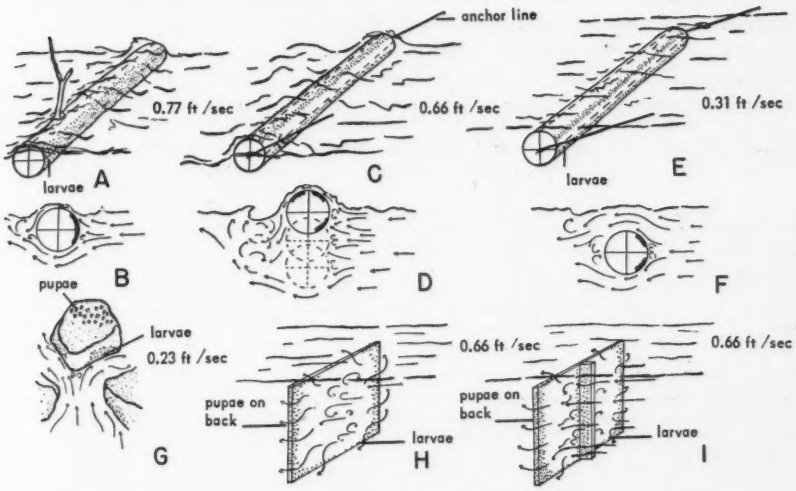


FIG. 10. The patterns of attachment of larvae on various surfaces, showing their relationship to turbulent and streamline flows of the water: A and B, on white birch log lying across the stream just below the water surface; C and D, on white birch log anchored across the stream at a constant depth of 6 to 8 inches below the surface; G, on stones; H, on flat wooden square fixed at right angles to the current; I, on flat wooden square fixed at right angles to the current and bisected on the upstream surface by a 2-in. batten.

The heaviest larval infestations were found in shallow rill sections of permanent streams, such as the narrow outlets of lakes and ponds and the slow-flowing sections of rivers. The water in these parts warms rapidly in the spring. In June, the maximum daily temperatures of these waters were 55° to 60° F, in July 70° F, in August 60° F, and in September 50° F. Boards, logs, concrete sluices, and dams were the next most heavily infested areas. In forest streams and in cool spring-fed trickles, the general level of infestation was low and *P. hirtipes* was the important biting species in these areas. In some streams the temperature never exceeded 50° F and the development of the larvae was delayed. No larvae were found in streams with a sandy or muddy bed, or in deep, sluggish streams with steep banks. *C. invenusta*, *S. corbis*, *P. hirtipes*, and *P. multidentatum* were the only species found in water deeper than 18 inches.

The pH of the black-fly streams at Baie Comeau was between 7.0 and 7.5. All heavily infested waters were well oxygenated. Larvae were found only in small numbers in water polluted by decaying vegetation, while waters polluted by garbage or sewage were apparently unsuitable.

Black-fly larvae are positively phototropic (1, 41). A larval preference for light-colored rocks and submerged white birch logs was found in our

studies. The results of one experiment with painted and unpainted surfaces of a spruce log are given in Table IV. Simuliid larvae attach in only very small numbers to jack pine logs. This and the small number that attached to the unpainted section of the spruce log is probably due to the repellent effect of the resinous exudates of coniferous wood.

TABLE IV

Total numbers of black-fly larvae and pupae found on sections of a spruce log, of three colors,* in Brisson Creek in Baie Comeau area, Quebec, 1954

| Date | Mean current ft/sec† | White | | Black | | Unpainted | |
|---|---|---|---|---|---|---|---|
| | | Larvae | Pupae | Larvae | Pupae | Larvae | Pupae |
| June 30 | 0.27 | 880 | 0 | 30 | 0 | 52 | 0 |
| July 5 | 0.31 | 153 | 16 | 6 | 2 | 3 | 0 |
| July 12 | 0.31 | 52 | 7 | 0 | 0 | 1 | 0 |
| July 26 | ‡ | 257 | 0 | 78 | 0 | 89 | 0 |
| Aug. 2 | 0.57 | 103 | 0 | 11 | 0 | 7 | 0 |
| Total | | 1445 | 23 | 125 | 2 | 152 | 0 |
| Percentage of total | | 83.9 | 92 | 7.2 | 8 | 8.8 | 0 |

*A 4-ft log divided into nine equal segments alternately white, black, and unpainted placed in the creek where the flow was laminar and the log constantly broke the surface.
†Measured by Gurley flowmeter.
‡No record.

Associated with the larvae on rocks were larvae of net-winged midges (*Blepharoceridae*), May-fly larvae (*Heptagenia* spp.), caddis-fly larvae (*Goera* and *Ryacophila* spp.), and larvae and pupae of the midge *Tanytarsus*. The stone-fly, *Perla immarginata*, and caddis-fly larvae of the genus *Hydropsyche* were found to feed on simuliid larvae. Predation by caddis-fly larvae has been reported by several workers (23, 26, 29). Twinn (44) has discussed the role of fish and insect predators, as well as fungi and parasites of black flies. The gut contents of several speckled trout, *Salvelinus fontinalis*, from Brisson Creek were examined and found to contain many larvae and pupae of black flies. Cameron (3) and Twinn (44) both reported that fish, particularly the sucker fish, *Catostomus commersonii* (Lacepède), are predators of the larvae of *S. arcticum*. The only parasites of larvae found at Baie Comeau were nematodes of the family Mermithidae. Parasitized larvae were readily distinguished by their swollen and white abdomens. An increase in the incidence of parasitized larvae was noted in July but was always less than 1% of the total population.

*The Effect of Floods, Flushes, Logging, and Fire on Black-Fly Populations*

It was observed that during floods the larvae were washed away, except those attached at the extreme tips of grasses that rested parallel to the current direction. After a few days of heavy rain, the larval populations were also noticeably reduced. On July 11, 1955, after heavy rains, Papanachois Falls, which previously supported a large population of larvae, was almost completely cleared. The remaining larvae were attached to trailing vegetation close to the banks. Similarly, floods altered the distribution of larvae in a

stream. Certain rill sections in Brisson Creek were almost completely cleared of larvae after very heavy rains, and larvae were found in sections of the stream that previously contained no larvae. On these occasions larvae were flushed through lakelike sections of the streams.

The effects of opening a sluice gate in a stream in which larvae were abundant, were observed. It was thought that partial control of black flies might be achieved by using the existing hydrological facilities of the pulpwood limits. The results of artificially flushing Brisson Creek are shown in Fig. 11.

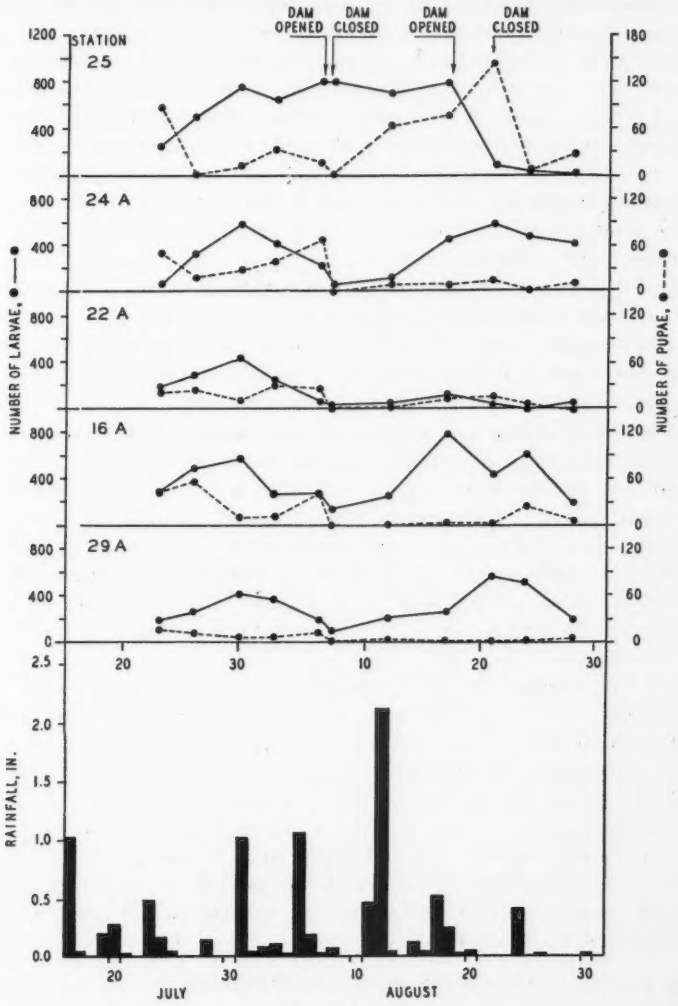


FIG. 11. Comparative levels of populations of larvae and pupae, determined by 5-minute stone counts, at five stations on Brisson Creek, July 23 to August 29, 1954.

Larval populations were assessed by either stone or pole count methods. For the former, three observers each counted the number of larvae attached to stones that they removed from the stream bed over a period of 5 minutes. For the pole counts, pulpwood logs, 4 ft in length and painted white, were placed in streams, and anchored by rope to the shore so that they were submerged 4 to 6 inches beneath the water surface. The number of larvae attached to the pole was counted. In interpreting the results of these experiments, the following points should be kept in mind: during flooding, conditions for attachment in one section of the creek were adverse but other sections, previously unfavorable, were now found to contain large numbers of larvae; larvae became attached to floating grasses on the water surface rather than on stones; the experiment was carried out in August after the maximum period of infestation; and, the rainfall during the experiment altered the effect of the post-flood desiccation period, which it is thought was an important part of control by artificial floodings. The results showed clearing of the streams in some sections and an increase in the number of larvae in others. Artificial flooding altered the distribution but did not control the larval populations to any significant degree.

Dampf (6) described methods of combating onchocerciasis in Mexico by the control of larvae through the periodic deflection of streams into a system of receptacles of calm water. These methods would be impractical in Canada. Further, the dams and their spillways create excellent oviposition sites for certain species, including the major pest *S. venustum*.

Logging drives in the spring and summer completely cleared streams of larvae and pupae (Fig. 5). The combined effects of a sudden rise in water level and the constant disturbance of the stream bed and bank by the logs made attachment of larvae impossible, either on the substratum or on trailing vegetation. Two years at least were required to restore the larval population to pre-logging levels. Although it might be thought that the population of black flies in the area is reduced by the logging drive, only selected streams are used and the elimination of these as effective breeding areas made no noticeable change in the adult population.

During the summer of 1955, observations were made in an area burned over in 1952. There was little new vegetation and the area was exposed to the full strength of wind and sunlight. Black-fly activity and larval populations in this type of country, even though there were many suitable watercourses, were very light. The presence of trees and border vegetation in which the flies shelter, appears to be a primary requirement for black-fly activity in forested areas. A reduction in adult population was noted after the fires in late July, 1955. Enormous numbers of flies were driven out of the area by the smoke. Three weeks after the fire had passed over an area, no adult black flies were found or larvae recovered from the streams.

*Mating, Feeding, Ovarian Development, and Oviposition*

Mating was observed in the field in only two species, *S. decorum* and *C. dacotensis*. Males generally emerged 1 to 2 days before the females and were

found resting on border vegetation and stones. Beneath the dam at Lac la Loutre, first-generation females of *S. decorum* emerged and mated within 12 hours while crawling on the stones and boards of the dam and, by 48 hours, were observed ovipositing at the same sites, just above the water level and in the spray zone.

The only species of black flies found to feed on the blood of humans at Baie Comeau were *P. hirtipes*, *S. venustum*, *S. parnassum*, and *S. tuberosum*. Females as well as males fed on the nectar of flowers. Blueberry flowers were particularly favored by *Simulium* sp. M.

A very successful method of capturing males was by sweeping the vegetation bordering the roads. Male swarms of *P. hirtipes*, *Simulium* sp. M, *S. venustum*, and *S. vittatum* were observed in the field.

Oviposition by six species was observed. It occurred from the late afternoon until twilight (i.e., 1800 to 2100 hours) of bright sunny days. On overcast days, even greater numbers were observed ovipositing as early as 1400 hours. *S. venustum* and *S. vittatum* deposited their distinctive egg masses on floating vegetation below lake outlets, in pools, or where slow-moving sections of rivers changed to rapids. *S. tuberosum* also deposited eggs in these locations but directly into the water and singly or in small groups. *S. decorum* deposited eggs in irregular masses on dam structures within the spray zone, and *Simulium* sp. M similarly on rocks intermittently flooded by the surge of waters in cascades and waterfalls. *S. decorum* and *S. vittatum* did not, as was reported by Davies and Peterson (10), oviposit in flight. *P. hirtipes* deposited eggs singly while in flight over the crests of falls and cascades, or in swiftly flowing sections of the watercourses. Figures 7–9 illustrate typical oviposition sites and egg masses.

*Longevity and Flight Range*

The adult black flies were capable of living in nature for rather long periods. *P. hirtipes* was collected in August, at least 30 days after the last emergence was observed. Small numbers of *S. venustum* probably survived for similar periods, indicating that adults of one generation may survive until the following generation emerges in any one season. The first experimental data for longevity in black flies was reported by Dalmat (4). He used aniline dye markers and, in Mexico, obtained times varying from 3 to 85 days. In contrast to this is the short time black flies survive in captivity. Although there are many records of survival times of 16 to 20 days, they refer only to a few flies of an initial number of hundreds (2, 5, 9, 20, 46). We followed the methods of Davies (9) and made numerous modifications, but were unable to maintain adults of *S. venustum* and *S. vittatum* for longer than 2 days in captivity. The establishment of a self-perpetuating colony is one of the important problems to be solved by students of black flies. Fredeen (19) has obtained promising results with laboratory rearing techniques.

*S. venustum* and *P. hirtipes* were collected, in forested areas, 5 miles from the nearest breeding waters. The range of the dispersal was much greater in recently cutover areas.

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# SOME FACTORS AFFECTING DIAPAUSE IN THE EUROPEAN CORN BORER, OSTRINIA NUBILALIS (HBN.) (LEPIDOPTERA: PYRALIDAE)[1]

J. A. MUTCHMOR[2] AND W. E. BECKEL[3]

## Abstract

At 65° F, the percentage of larvae of the European corn borer entering diapause increased from practically 0 to 95 as the photoperiod was increased from 0 to 9.5–14 hours of light per day, and decreased sharply to a minimum of 3 as photoperiod was increased to 16 or more hours of light per day. Rearing and studies on oxygen consumption revealed that, to be most effective, exposure to factors inducing diapause must begin before the second day of the final instar, and must continue for a period equivalent to the duration of the final instar at 65° F. Exposure during any single instar but the last was not effective in inducing diapause.

## Introduction

A major change has taken place in the seasonal history of the European corn borer, *Ostrinia nubilalis* (Hbn.), since it was reported in southwestern Ontario in 1920. Until about 1941 the borer was primarily univoltine. In the ensuing years, there has been an increase in the relative size of a second generation so that now it constitutes an important part of the borer population. The transition from one to two generations per year and its economic consequences in Ontario were described and discussed by Wressell (11). Lees (7) reviewed the literature on voltinism in the borer, its geographic distribution in Europe and North America, and the transition from one to two generations in some areas of the United States. Arbuthnot (1) associated the number of generations of the borer in various parts of the world with regional differences in temperature and precipitation.

The second borer generation can occur only if larvae of the first generation fail to enter diapause in the final instar. Despite its importance, little has previously been known of the factors responsible for the induction of diapause in the corn borer. O'Kane and Lowry (9) indicated that the factors were environmental; larvae from eggs hatching early in the season completed development and produced a second generation, whereas those from eggs hatching later in the summer entered diapause. Babcock (2) reported that the larvae were very sensitive to changes in contact moisture during dormancy; if there was no compensation for desiccation before the termination of diapause, the number of one-generation descendants tended to increase. Kozhanchikov (6) found temperature to be important in inducing diapause; most borers kept at temperatures between 14° and 24° C entered diapause, but no diapause occurred at 26.1° to 34° C. Mutchmor and Beckel (8),

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in a preliminary report, showed that photoperiod was of primary importance in inducing diapause in laboratory-reared larvae. This paper presents in detail the results of laboratory studies on the role of photoperiod and other factors in induction of diapause in corn borer larvae.

## General Methods and Materials

The borers were laboratory-reared descendants of larvae dissected from corn grown near Harrow, Ontario. Under the laboratory conditions the borers were multivoltine; eight successive generations were reared without interruption by diapause. Stocks of larvae were reared, as individuals, in 1-dram shell vials under continuous light at 85° F. Each vial contained a cylinder of food medium about $\frac{3}{8}$ in. long and $\frac{1}{4}$ in. in diameter, and was tightly stoppered with a cotton plug. The food was similar to that described by Beck *et al.* (3) but a different microorganism inhibitor (methyl *p*-hydroxybenzoate) was used. The larvae were transferred to vials containing fresh food at 4- or 5-day intervals, or more frequently when necessary. Borers at the desired stages of development were withdrawn from the stock as required.

Failure to pupate was the criterion used to determine whether diapause had been induced by the various treatments. Most of the experiments were carried out at 65° F; at that temperature the duration of the final larval instar averaged 21 days. Larvae that had not pupated after 30 to 40 days in the final instar were almost always in diapause. Nevertheless, each larva was then checked for diapause by being kept at 85° F for 7 days, a period sufficient for completion of the final instar; larvae failing to pupate were then considered to be in diapause.

A 24-hour cycle of alternating light and dark phases was used to determine the relationship of photoperiod to induction of diapause. Two 15-w fluorescent daylight lamps placed in the roof of a 65° F constant temperature cabinet, 12 to 18 in. from the larval containers, provided the light. Certain of the photoperiods were controlled by a day–night clock switch wired to the light circuit. Other photoperiods were controlled manually. For their dark periods the larvae, each in a shell vial, were placed in 1-pt Mono cups (Continental Can Company of Canada Ltd., Mono Paper Division, New Toronto, Ontario). These cups had been coated on the inside with black latex paint and covered on the outside with heavy metal foil. In the light phases unmodified Mono cups were used. The positions of the containers in relation to the lamps were changed each day. Light intensity in the positions occupied by the Mono cups was approximately 12 ft-c. The larvae in their containers, of course, received considerably less light. Larvae were examined and transferred to fresh food only during light phases. However, those reared in continuous darkness were exposed to light during food changes for approximately 40 minutes each week.

Changes in the weight and oxygen consumption of larvae were measured in one of the experiments. The borers were weighed on a Roller–Smith

torsion balance. Oxygen consumption was determined immediately after the borers were weighed. Each larva was encased in a perforated gelatin capsule and placed in the side-arm of a Warburg flask with 3 ml of 10% potassium hydroxide in the center well. Bath temperature was controlled at 30° C. After a 15-minute equilibration period, the amount of oxygen consumed by each larva per hour was determined. The larvae were then returned to their rearing cabinets.

## Influence of Photoperiod on Induction of Diapause

Groups of larvae reared to the end of the fourth instar at 85° F in continuous light were transferred to various photoperiods at 65° F for 30 to 40 days or until they had pupated. Ecdysis, pupation, and mortality were recorded each day during light periods. Larvae reared in darkness were examined twice each week. Ten photoperiods were tested: 0, 4, 8, 8.5, 9.5, 12, 14, 16, 20, and 24 hours of light per day. Photoperiods of 0, 9.5, 14, and 24 hours of light per day were duplicated.

Figure 1 shows that the percentage of the surviving larvae entering diapause was determined by the photoperiod to which they were exposed during the final instar. Few larvae entered diapause when reared in continuous darkness or at photoperiods of 16 to 24 hours of light per day. The percentage that entered diapause increased as the photoperiod was lengthened from 0 to 9.5 hours of light per day, and was at a maximum over the range 9.5 to 14 hours of light per day. Mortality ranged from 0 to 43%, but relatively large mortality differences between duplicate tests did not produce large differences in the percentages of survivors entering diapause. For replicates at 0, 9.5, 14, and 24 hours of light per day, percentage mortalities were 10 and 43, 7 and 22, 0 and 12, and 2 and 16, respectively. Percentages of survivors in diapause were 7 and 0, 94 and 96, 92 and 87, and 8 and 5.

In a similar experiment in which larvae were transferred to 75° F and 0, 8, 16, and 24 hours of light per day, no larvae entered diapause.

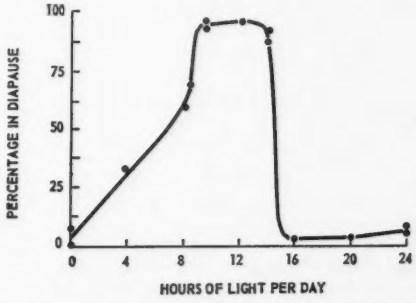



FIG. 1. Percentages of the European corn borer that entered diapause when larvae were reared in the final larval instar at 65° F and various photoperiods. The number of larvae per group ranged from 25 to 75.

## Influence of Time and Duration of Exposure to Diapause-inducing Factors on Induction of Diapause

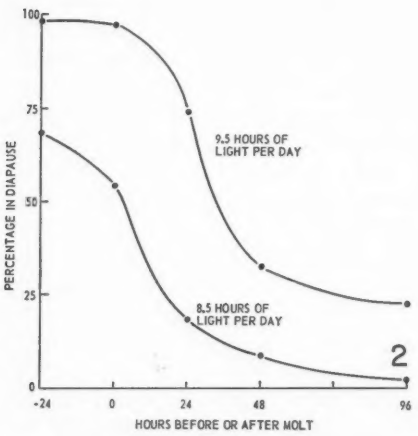
A series of experiments was performed to determine the stage at which diapause-inducing factors are most effective. Changes in weight and oxygen consumption during initiation of diapause were measured to supplement data from rearing experiments.

When groups of 50 or 75 larvae were reared in a single instar at 65° F and 8.5 hours of light per day and in the remaining instars at 85° F in continuous light, the percentages that entered diapause were as follows:

| Instar at 65° F | First | Second | Third | Fourth | Fifth |
|---|---|---|---|---|---|
| No of larvae | 75 | 50 | 50 | 50 | 50 |
| Larvae in diapause (%) | 0 | 0 | 0 | 4 | 71 |

Mortality ranged from 38 to 46%. Exposure to diapause-inducing conditions in any instar but the last had relatively little influence on induction of diapause.

To determine whether there is a period in the final instar during or before which the exposure to diapause-inducing factors is most effective, five groups of borers were reared at 85° F in continuous light and transferred at selected intervals to 65° F and 8.5 and 9.5 hours of light per day. One group was transferred approximately 24 hours before the molt to the final instar, the second before the head capsules had darkened after the molt, and the remaining three at 24, 48, and 96 hours after the molt. The number of larvae per



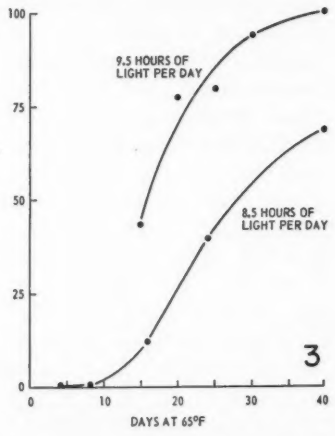




FIG. 2. Percentages of the European corn borer that entered diapause when larvae were transferred from 85° F and continuous light to 65° F and two photoperiods at various intervals before and after the molt to the last instar.

FIG. 3. Percentages of the European corn borer that entered diapause when larvae, reared at 85° F in continuous light, were transferred to 65° F and two photoperiods for various numbers of days after the molt to the last instar and then were returned to 85° F.

group ranged from 42 to 75, averaging 56; mortality varied from 0 to 8%, with an average of 5%. Figure 2 shows that there is no sharply defined critical period since diapause occurred in each of the five age groups tested. However, the diapause-inducing treatment was much less effective when exposure was begun after the larvae had been a day or more in the final instar.

The preceding experiments revealed that, to be most effective, diapause-inducing treatments must be started by the first day of the final instar. The optimum duration of such treatments was determined in the following experiments.

Larvae reared at 85° F in continuous light and transferred to 65° F and 8.5 hours of light per day shortly before the beginning of the final instar were returned to 85° F and continuous light at 4, 8, 16, or 24 days after the beginning of the instar. In a second experiment, larvae were transferred to 65° F and 9.5 hours of light per day and returned to 85° F at 15, 20, 25, 30, or 40 days after the beginning of the final instar. The average number of borers per group was 16 in the first experiment and 50 in the second. A value for larvae held for 40 days at 65° F and 8.5 hours of light per day, obtained in an earlier study, was used to complete the lower curve of Fig. 3. This figure shows that a comparatively long period at 65° F (30 to 40 days) is required to induce diapause in the maximum number of borers but, for many of the borers, 15 days are sufficient. The rate of the induction process itself does not appear to be influenced by photoperiod, since at both 8.5 and 9.5 hours of light per day the maximum levels of diapause were reached in about the same time.

Changes in weight and oxygen consumption of larvae entering diapause at 65° F were determined so that a more precise estimate could be made of the time required for induction of diapause. Determinations for 18 larvae placed at 65° F and 9.5 hours of light per day at the end of the fourth instar, and for 9 larvae that were left at 85° F as a non-diapause control, were made at 2- to 4-day intervals until they had pupated or had been in the final instar 30 days.

Weights and oxygen consumptions of the control larvae at 85° F and of three larvae that failed to enter diapause at 65° F increased until the larvae stopped feeding shortly before pupation. In contrast, the values for the 14 larvae that entered diapause reached maxima after the larvae had been in the final instar an average of 19 days (range: 16 to 27 days), having increased from 30 mg (range: 20 to 46 mg) and 31 $\mu$l. of oxygen per hour (range: 14 to 50 $\mu$l.) on the first day of the instar to 94 mg (range: 66 to 122 mg) and 108 $\mu$l. (range: 79 to 151 $\mu$l.). A very sharp decline in oxygen consumption then began so that on the 28th day of the instar it averaged only 21 $\mu$l. per hour (range: 3 to 50 $\mu$l.). A less spectacular loss in weight accompanied the drop in oxygen consumption. When larvae were at their maximum weights, the average oxygen consumption was 1.15 $\mu$l./mg/hr, whereas 8 days later it was only 0.26 $\mu$l. These values reflect the difference in the rate of decline of weight and oxygen consumption.

If the sudden reduction in oxygen consumption and weight coincides with the establishment of diapause, then the time required for the induction of diapause in individual borers can be sharply defined. Depending on the larva, the time may range from at least 16 to 27 days. Such variation is consistent with the results of the foregoing rearing experiments.

## Discussion

Photoperiod influences the onset of diapause in a number of Lepidoptera (7). This study shows that such is the case for larvae of the European corn borer. The influence of photoperiod on the induction of diapause is primary; since the larvae were reared on a non-living medium, there was no possibility for photoperiod or temperature to influence diapause indirectly, through changes induced in a food plant.

Dickson (5) reported similar results with comparable photoperiods in the induction of diapause in the oriental fruit moth, *Grapholitha molesta* (Busck), and in addition found that the absolute durations of both the light and the dark phases were important in determining diapause. To induce diapause in appreciable numbers of larvae, the dark phase had to be between 11 and 16 hours and the light phase between 7 and 16 hours. Combinations of light and dark phases in which either component was outside these limits largely prevented diapause. If similar conditions govern diapause in the borer the limits are evidently somewhat broader, since a light phase of only 4 hours alternating with a dark phase of 20 hours allowed 32% to enter diapause (Fig. 1).

Way and Hopkins (10) found, with *Diataraxia oleracea* (L.), that diapause-inducing conditions were most effective when applied about 2 days before the end of the penultimate instar and maintained until the fifth day of the final instar. Such conditions are most effective for induction of diapause in the corn borer when exposure begins no later than the first day of the final instar and is continued for a relatively long period. At 9.5 hours of light per day, 30 days at 65° F were required to induce diapause in 94% of the larvae. Reduction in weight and oxygen consumption of larvae entering diapause at 65° F began 16 to 27 days (average of 19 days) after the beginning of the final instar. When a group of larvae were reared at 65° F, under a diapause-preventing photoperiod, the duration of the final larval stage was 12 to 30 days (average of 21 days), a range comparable to that required for induction of diapause. Therefore, the time required to induce diapause at 65° F is roughly equivalent to the duration of the final larval stage at that temperature.

Way and Hopkins (10) found that diapause in *D. oleracea* was prevented by high temperatures, but that low temperatures promoted induction of diapause even when diapause-preventing photoperiods were used. On the other hand, Dickson (5) showed that *G. molesta* enters diapause only at intermediate temperatures. The corn borer was studied mainly at 65° F, at which temperature diapause is clearly dependent on photoperiod. Diapause was not induced at 75° F with any of four photoperiods. Though no formal experiments

were conducted at 55° F, diapause was readily induced at that temperature when larvae were exposed to a diapause-inducing photoperiod from the beginning of the final instar. Evidently diapause in the borer is prevented by high temperatures, regardless of the photoperiod, but not by low temperatures.

This study provides some insight into the occurrence of diapause in the field. If the sensitivity of the corn borer to illumination is of the same order as that of other arthropods (7), then the effective photoperiod under natural conditions must correspond closely to day-length. Figure 1 indicates that the critical day-length for the borer is between 14.5 and 15 hours. At latitude 43° 40′ day-length, as measured from sunrise to sunset, exceeds 14.5 hours from the second week of May until about the last week of July. During this period, therefore, day-lengths are diapause-preventing. For the remainder of summer and fall, they are diapause-inducing. The results of outdoor rearing studies at Chatham, to be reported in detail in a future paper, show that most borers entering the final instar later than the third week of July enter diapause, whereas those entering the final instar earlier in the season pupate. Thus, the seasonal transition from borers completing development to those entering diapause corresponds to the change from diapause-preventing to diapause-inducing day-lengths. Temperatures during August, when diapause occurs and when diapause-inducing day-lengths prevail, are obviously not high enough to prevent diapause. It is less clear whether diapause in July is prevented by diapause-preventing day-lengths or by higher temperatures. That temperature may be less important than day-length in preventing diapause in July is indicated by the fact that in the Lake St. Clair, Lake Erie, and Niagara Peninsula regions of southwestern Ontario, the maximum, minimum, and mean temperatures for July average only about 2° higher than the corresponding temperatures for August (4). In any case, since photoperiod is a factor, the transition to diapause-inducing day-lengths will occur, over a 1- or 2-week period, at about the same time each year. The importance of larval age in the induction of diapause is evident. Larvae that have been more than a day in the final instar when the change to diapause-inducing day-lengths occurs will complete development and may produce a second generation. Younger larvae, reaching the final instar after the change in day-length, will enter diapause and will not pupate until the following spring. Many larvae that would otherwise complete development too late in the season to produce a successful second generation thus are saved by diapause. Furthermore, the relative size of the second generation will, in large measure, be determined by factors favoring the early development of first-generation larvae to the final instar.

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# NOTES ON THE PATHOGENICITY OF SERRATIA MARCESCENS BIZIO FOR THE COCKROACH BLATTELLA GERMANICA L.[1]

A. M. HEIMPEL[2] AND A. S. WEST

## Abstract

*Serratia marcescens* Bizio sporadically caused mortality in cultures of the German cockroach, *Blattella germanica* L. Extensive feeding tests showed this bacterium is not normally pathogenic, *per os*, for the insect. The $LD_{50}$, by injection, for the cockroach is approximately 38,000 bacteria per insect. The possible modes of invasion of the insect gut are discussed briefly.

## Introduction

*Serratia marcescens* Bizio is a small, Gram-negative, bacterial rod of the family Enterobacteriaceae. References to the organism appear frequently in the literature, and possibly because of the distinctive appearance of the red colony it has been used in studies of the survival of bacteria in the insect digestive tract. According to Steinhaus (4) it has been shown that *S. marcescens* can be ingested and retained for varying lengths of time, by several different insects. Steinhaus (5) has summarized reports on the virulence of *S. marcescens* for a variety of insects. More recently Miss June Stephens of the Entomology Laboratory, Belleville, Ontario (personal communications), has isolated strains of the bacterium from diseased larvae of the codling moth, *Carpocapsa pomonella* (L.), when it caused severe mortality in a rearing stock of that insect. Steinhaus and Bell (6) reported that the rice weevil, *Sitophilus oryza* (L.), the granary weevil, *Sitophilus granarius* (L.), and the confused flour beetle, *Tribolium confusum* Duv., were all slightly susceptible to *S. marcescens*; they found that the Angoumois grain moth, *Sitotroga cerealella* (Oliv.), was not susceptible. Heimpel (3) reported that a strain of *S. marcescens*, isolated from *C. pomonella*, was pathogenic in varying degrees for several species of sawflies. *Pristiphora erichsonii* (Htg.), *Neodiprion lecontei* (Fitch), and *Neodriprion pratti banksianae* Rohwer were the most susceptible (55 to 60% mortality); *Neodiprion swainei* Midd. and *Nematus ribesii* (Scop.) were slightly less susceptible (40 to 50% mortality).

Chattopadhyay and Mukherjee (1) reported isolation of a strain of *S. marcescens* from *Agrotis ypsilon* Rott. The investigators described the organism but did not mention its pathogenicity for the insect.

Experience over a period of years has shown that *S. marcescens* may appear sporadically in cultures of a variety of insects and may be highly pathogenic, causing complete mortality of particular groups of insects. This sporadic occurrence has been observed in cultures of several species of *Droso-*

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*phila*, the roach *B. germanica*, the codling moth, *C. pomonella*, and the red-headed pine sawfly, *N. lecontei*. In all these cultures rearing conditions were probably highly favorable for the multiplication of the organism; the accidental introduction of *S. marcescens* wiped out a colony of the red-headed pine sawfly larvae consisting of thousands of last-instar larvae crowded in an outdoor cage.

Frequent attempts were made to infect *B. germanica* cultures with *S. marcescens*. Several strains of the organism, all isolated from insects killed by the organism, were used. Broth cultures were introduced on saturated cotton pads; dog-food cubes were soaked in broth cultures and fed to the roaches; and roaches were sprayed with broth cultures. In addition roaches recently killed by the organism were introduced into colonies. Not one of these attempts succeeded in establishing an infection even when the organism survived and multiplied on the roach food. However, during these attempts, an infection would very occasionally exterminate an untreated colony. It was shown in such cultures that the organism had caused a septicaemia in the hemocoele of the roach. *S. marcescens* was isolated in these cultures, but feeding and injection tests failed to show any special pathogenic qualities.[3]

Thus it became apparent that the pathogenicity of *S. marcescens* for the roach, and presumably for other insects, was dependent on factors which permitted invasion of the hemocoele. Presumably such factors might be roach-dependent or organism-dependent.

## Methods and Materials

Rearing methods closely followed those outlined by Heal (2). The roach cultures were maintained at 27° C and the age of all individuals was known to within 7 days.

Gravid females were placed in a 5-in.-square screen hatching cage which was in turn placed in a rearing jar. The rearing jar was a circular battery jar approximately 12 in. high and contained four fiberboard platforms one-half inch apart. Water, and food in the form of dog biscuits, were supplied to both the hatching cage and rearing jar. As the eggs hatched, the nymphs passed through the screen mesh from the hatching cage to the rearing jar.

The hatching cage was transferred to a fresh jar every week. Approximately 400 roaches were reared in a jar. For handling outside the jar, the roaches were anaesthetized with carbon dioxide.

Individual rearings were maintained in shell vials, each supplied with a brick of food and water. Ten per cent Mycoban was used to inhibit fungus growth on the food. For some of the tests the roaches were kept in 250-ml Erlenmeyer flasks.

The injections were made with a Dutky micro-injector fitted with a $\frac{1}{4}$ ml syringe with a No. 30 gauge needle. Serial dilutions were made of a 24-hour

[3]The authors are indebted to the former Miss Margaret Campaigne for much of the laboratory assessment.

broth culture of *S. marcescens* (isolated from the codling moth), incubated at 27° C. Injections were given under the coxa of the left metathoracic leg. Approximately 0.006 ml of fluid was injected per roach. In most experiments, aliquots of each dilution were injected into 10 to 20 roaches. Drop-plate counts of the injected bacterial suspensions established that undiluted cultures contained approximately $1.4 \times 10^9$ bacteria per milliliter under these conditions. Controls for the inoculations were obtained from screening tests carried out on 166 stock cultures representing several families of bacteria. Fifty of these cultures, other than *S. marcescens*, were injected ($5.4 \times 10^5$ to $1.3 \times 10^6$ bacteria per roach) into some 500 roaches without deleterious effect upon the test animals. Likewise, injection of water or buffered gelatin solution had no effect.

## Results

The results of the initial injection experiments are given in Table I. For these tests both male and female roaches were used. The adult age was between 1 and 2 weeks. The roaches were held individually in 1 in.×3 in. shell vials following injection.

Most insects injected with $8.4 \times 10^4$ bacteria ($10^{-1}$ dilution) were dead within 24 hours. For the other dilutions very few deaths occurred before 7 days. After 11 days the incidence of mortality dropped abruptly; therefore, an arbitrary time limit of 11 days was set as the end. The $LD_{50}$ of *S. marcescens* for the cockroach then lies somewhere between 8400 and 84,000 bacteria per roach, by interpolation, probably in the vicinity of 38,000 bacteria per roach.

Two hundred and six survivors were kept under close observation until death. After the initial 11-day period 38 roaches were recorded as missing, were crushed during handling, or no diagnosis was made. Of the remainder, 28 females and 60 males contained *S. marcescens* upon death; however, their deaths were apparently normal as they occurred at approximately the same rate and time as the deaths of female and male normal, uninjected roaches. In the above experiment the bacterium survived in certain roaches up to 330 days without apparent harm to the host or the bacterium.

TABLE I

Mortality observed in 11 days after injection of 1- to 2-week-old roaches with serial dilutions of *S. marcescens* broth cultures, incubated at 27° C for 24 hours

| Injected dosage (viable bacteria) | Number of insects | Number dead | Percentage killed by *S. marcescens* |
|---|---|---|---|
| $8.4 \times 10^4$ | 138 | 135 | 98 |
| $8.4 \times 10^3$ | 134 | 48 | 34 |
| $8.4 \times 10^2$ | 115 | 35 | 29 |
| $8.4 \times 10^1$ | 95 | 20 | 17 |
| Total | 482 | 238 | |

TABLE II

Mortality of cockroaches of various ages injected, in lots of 10, with serial dilutions of *S. marcescens* broth cultures

| Injected dosage (viable bacteria) | Age, in days | | | | |
|---|---|---|---|---|---|
| | Nymphs | | Adults | | |
| | 42 | 61 | 23 | 56 | 107 |
| $8.4 \times 10^4$ | 10 | 8 | 10 | 8 | 10 |
| $8.4 \times 10^3$ | 0 | 0 | 0 | 1 | 4 |
| $8.4 \times 10^2$ | 1 | 1 | 0 | 1 | 1 |
| $8.4 \times 10^1$ | 1 | 0 | 1 | 0 | 0 |

*The Effect of Age on Susceptibility to S. marcescens*

Based on small numbers, a comparison of age groups with respect to susceptibility shows no apparent significant differences among nymphs and adults of various ages (Table II).

## Discussion and Conclusions

*Serratia marcescens* is not normally pathogenic, *per os*, for the German cockroach, and the $LD_{50}$, by injection, is approximately 38,000 bacteria per insect. Obviously the virulence of this bacterium for the insect is low. In any event, the special conditions that make "spontaneous infections" possible are not associated with any special pathogenic attributes of the strains of *S. marcescens* tested here. Further, in these experiments, the humidity and temperature had no significance, since these conditions were controlled throughout. It might be postulated that the state of the insect is responsible for the sudden changes in susceptibility.

The use of *S. marcescens* as a control organism in feeding trials to test the virulence of other species of bacteria for the German cockroach can be recommended. It is admirably suitable for such a purpose, since it has a distinctive colony color, and is easily distinguished in the insect or upon replating on agar. The bacterium also eliminates the probably subnormal insects by causing a septicaemia, and in so doing gives an estimate of the number of subnormal insects in the test population.

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# FOOD AS A CONTROL OF A POPULATION OF WHITE-FOOTED MICE, PEROMYSCUS LEUCOPUS NOVEBORACENSIS (FISCHER)[1]

J. F. BENDELL

## Abstract

Mice were introduced to an island before and after food was supplied in excess of food consumed. The population of mice on the experimental island with food in excess is compared with populations of mice on the same island before the addition of food, on an adjacent island, and on the mainland. Before food was supplied in excess, death rate (death and dispersal) greatly exceeded birth rate and the population failed. After food was supplied in excess death rate of the second introduction decreased and population increased to greatly exceed the density of mice on the control island.

The increased density of mice on the experimental island is the result of a decreased death rate, particularly in mice from birth to approximately 1 month of age. Increased birth rate may also be a factor. Food supply regulates the abundance of white-footed mice by affecting death rate and possibly birth rate. The most important effect of food supply is on the survival of young from birth to approximately 1 month of age.

Evidence that intraspecific strife for space occurs in relatively dense population is presented. Intraspecific strife for space may regulate population of mice beyond the level of abundance determined by the supply of food.

## Introduction

It seems obvious that a supply of food is an important factor affecting the welfare of animals in nature. In a recent book, Lack (23) concludes that food supply is of major importance in the control of abundance of many vertebrates. The work reported here is an analysis of the effect of food supply on populations of white-footed mice.

Our object was to find if food supply determined the abundance of mice, and if so, how food supply affected abundance. Moreover, assuming that food supply does limit population, an additional objective was to find how population is controlled beyond the limits of abundance determined by the supply of food.

Recently animal ecologists have resolved some of the difficulties of working with natural populations of rodents by experimenting with penned populations under a variety of conditions (Clarke (10), Louch (24), Southwick (29), Strecker and Emlen (32), and others). The approach to the present problem is an extension of this method. The experimental manipulation of the environment of mouse populations was done under natural conditions and with *P. l. noveboracensis* as the experimental animal.

In theory our method was to select two islands identical in habitat and populations of mice. One island is the experimental area and to this island food is added in excess. The second island serves as a control. The mainland provides an additional area of reference. It is argued that differences

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between populations of mice on the experimental and control areas are the direct or indirect effect of food supply on population.

In actual practice it was not possible to find two islands identical in habitat and populations of mice. This was not regarded as an obstacle, for the natural differences between islands were exploited to strengthen the results of the experiment.

## Materials and Methods

*The Study Area*

The experiment began in May of 1953 at Lake Opinicon in Eastern Ontario, and ended in February of 1957. Winter work was carried on from Queen's University at Kingston.

The study area is within the Great Lakes – St. Lawrence Forest Region, and Algonquin–Laurentides section of Halliday (16). The terrain is rough but without elevations of more than a few hundred feet. Numerous lakes and islands of various shapes and sizes occur throughout the area. The land is farmed and grazed so that the vegetation is a mosaic of fields and wood lots.

Two adjacent islands in Lake Opinicon were selected for the experiment (Fig. 1). Mice were also sampled from wood lots on the mainland. The islands and wood lots are similar in that they support predominantly hardwood forests on thin sandy soil. The bed rock of the area is mainly coarse, white, crystalline limestone.

Both islands are more or less dome-shaped, and rise from steep and rocky banks to relatively flat central plateaus. Each island is bordered by a belt of mixed coniferous and deciduous trees. Above this belt, and over most of the islands, the trees are predominantly deciduous. The shrub layer reflects the overstory. Important elements of the forest floor of both islands are debris, broken rock, fallen logs, and sedge.*

The islands are different in that one is 3.2 acres, the other 11.3 acres in size. The large island supports approximately twice the density of trees, shrubs, and vegetation of the forest floor that occurs on the small island. Significantly, the small island did not support a natural population of mice. A natural population of mice occupied the large island throughout the study.

Differences among islands and mainland in terrestrial vertebrates that might affect the welfare of mice are difficult to assess because of the secretive and transient nature of these animals. From time to time we captured and observed, on both islands, pilot black and milk snakes (*Elaphe obsoleta* and *Lampropeltis triangulum*), short-tailed shrew (*Blarina brevicauda*), meadow vole (*Microtus pennsylvanicus*), and red and grey squirrels (*Tamiasciurus hudsonicus* and *Sciurus carolinensis*). On the small island we captured or observed skunk (*Memphitis memphitis*) and house mouse (*Mus musculus*), and, on the large island, chipmunk (*Tamias striatus*) and long-tailed weasel (*Mustela frenata*). The weasel appeared on the control island in the last

*A detailed description of the vegetation of the islands and habitat selection of the white-footed mouse will be published later.

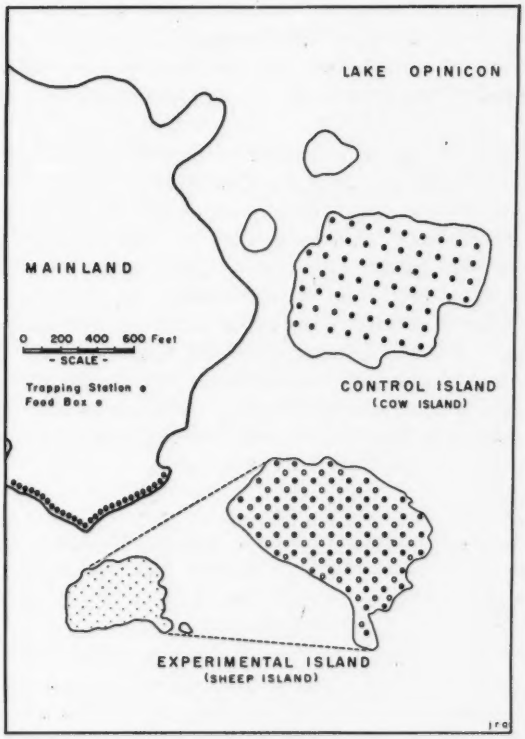



FIG. 1. Map of the experimental and control islands and a portion of the mainland.

summer of the work. This is the only terrestrial vertebrate that we can connect with the experiment and it is mentioned again later.

Since the large island had a resident population of mice and apparently better habitat for mice as indicated by a relatively dense stand of vegetation, it was used as the control island. The small island, without a natural population of mice and with relatively sparse vegetation, served as the experimental island. The choice of islands, while inevitable, fitted into the experimental plan. If populations could be made to respond to the addition of food to the experimental island then the response is made more meaningful by the previously poor and empty habitat.

*The Handling of Mice*

The populations of mice on the two islands were studied by live trapping. Animals were individually tagged with a numbered metal fingerling tag applied to one ear. Most data obtained from island mice were time and place of capture, sex, age, breeding condition, and in some cases weight.

The mainland population of mice was sampled by live and dead traps. Mainland mice were used as introductions and to start and augment a colony of approximately 100 animals that was maintained over the time of study. The mainland and colony mice provided supplementary data on the biology of the species.

Mice were autopsied fresh, or placed in capped bottles and deep-frozen, or preserved in 10% neutral formalin and examined later. Data regularly obtained on autopsy were weight to 0.1 g, total length and head–body length to 0.1 cm, age from weight and pattern of molt, weight of reproductive organs (in males the testes, epididymides, vasa deferentia, and vesicular glands; in females the ovaries, uteri, and vagina) to 0.1 g, number of recent and old corpora lutea, number of visible embryos, and weight of the paired adrenal glands to 0.1 mg. The last determination was made on an electrical balance. Corpora lutea were identified after Ironside (21) from prepared slides of specimen ovaries. Subsequently, corpora lutea were counted in whole ovaries. In addition to the above, a smaller sample of mice from the islands and mainland was examined for visceral parasites.

*The Supply of Food*

In the course of the experiment, food was supplied to the experimental island in excess of food consumed. The food was wheat, which was distributed in boxes placed evenly over the island (Fig. 1). Each food box contained, when full, approximately a cubic foot of grain.

A food box consisted of a square wooden box with a lid of wood, and asphalt roofing. The boxes were painted with a waterproof asphalt paint. Two pieces of wood formed a shallow "V" near the floor of each box. A slit between the lower edges of each piece of wood allowed the grain to flow onto the floor. Mice obtained access through slits in each side of the floor and parallel to the slit between the two pieces of wood. A strip of wood on the inner edge of each access opening prevented the leakage of grain. Dry grain ran well in a food box and formed a small pile on the floor.

Food boxes were set on wooden legs or nailed to the sides of trees approximately 2 ft above the forest floor. This was to keep the boxes dry and free from deep snow. Generally, branches were piled about food boxes to increase their accessibility.

No attempt was made to measure the depletion of food. Mice certainly made use of the food as indicated by the depletion of grain, mouse droppings, and husks of grain on the floor of the boxes, and tracks in the snow about the food boxes. In one case a câche of wheat was found in the nest of a mouse.

*Traps and the Relative Effectiveness of Traps*

Virtually all data on mice on the islands and mainland are from animals caught in traps. Three types of trap were used: the common break-back type of dead trap, and the Sherman, and tunnel live traps. Our Sherman live traps were made of galvanized iron and are 3 in. in height and width and 12 in. in length. The door at the end of the trap is a frame with a metal

grille. The tunnel traps were also made of galvanized iron and measure 2¼ in. in height, 2 in. in width, with a length of 7½ in. The tunnel trap consists of a treadle-trap-door and lock mechanism within the tunnel, and a holding can which receives the tunnel. The trap is without springs.

All traps were baited with peanut butter and oatmeal mixed to the consistency of soft putty. Fresh bait was placed on the traps after each night of trapping. In addition to bait, fox cubes, grain, and cotton wool for warmth were placed in live traps.

The three types of traps were tested against one another on the mainland to find their relative effectiveness in catching white-footed mice. In these tests a dead trap and a tunnel, or a tunnel and a Sherman were set together at approximately 30-ft intervals in lines through hardwood wood lots. In one test, Sherman and tunnel traps were placed side by side and run for an equal number of trap nights. The Sherman traps caught 19 mice; the tunnels 29. The difference in captures is not statistically significant (chi-square, $P > 0.05$), therefore Shermans and tunnels were equally effective in taking white-footed mice.

In a similar test, dead traps and tunnel traps were set together. The dead traps caught 35 mice, the tunnels 43 mice. The difference in captures is not statistically significant (chi-square, $P > 0.05$), hence dead and tunnel traps were equally effective.

Finally, a pair of dead traps was set with a pair of Sherman traps in a 50-ft grid in the experimental island at the termination of the field work. Over the trapping period, 31 mice were caught: 15 in dead traps and 16 in Sherman live traps. The conclusion from these three sets of results is that our live traps of the tunnel and Sherman type, and dead traps were equally effective in capturing white-footed mice.

*Trapping Methods*

The trapping on the mainland was, for the most part, simply to catch mice. For this purpose dead traps were most frequently used. Almost all trapping was done in hardwood wood lots; however, some mice were taken in and about vacant dwellings. The general trapping procedure on the mainland was to place one or a pair of traps on the ground at intervals of approximately 30 ft to make a line of traps. The trap lines were not always straight and contained from 10 to 100 sets (one or two traps) of traps. In calculating trap nights, a set of two traps is counted as one and the number of mice taken by a trap line is calculated as the number of mice captured over the first 100 trap nights.

At times traps were placed in a square 1-acre grid with a pair of traps at each 30-ft intersection. Trap lines or grids were tended every morning of a trapping period, which lasted from 3 days to several months. One mainland trap line is shown in Fig. 1.

The purpose of trapping on the islands was to estimate, with as little disturbance as possible, the total number of mice. Total estimates of population are of critical importance in this study. Hence, some attention is given to

the method of trapping on the islands and several tests are applied to the trapping data to measure their value as estimates of population.

Stickel (31) concluded that the live-trap quadrat method of estimating populations of *P. leucopus* gave accurate results, and she recommended a 50-ft spacing between traps. The method of trapping on the islands was to cover each island with a grid of live traps (Fig. 1). On the experimental island, intersections of grid lines occurred at 50 ft. On the control island the intersections were 100 ft apart. Two live traps were placed side by side and opposed at each intersection of lines on both islands. A 50-ft spacing between trapping stations placed 63 sets for a total of 126 traps on the experimental island. Despite the high density of mice that appeared on this island, rarely more than 50% of the sets held a mouse in a given trap night, and rarely was there more than one trap in a set occupied. Hence, the number of traps on the experimental island was large enough to prevent the number of traps limiting the number of captures.

The 100-ft interval between sets on the control island placed 62 pairs or 124 traps (Fig. 1). The 100-ft interval was adopted as a compromise between coverage of the island and the time and traps that were available. Also, the difference between spacing sets 50 and 100 ft seemed unimportant compared to the difference between one and two traps at a set. It is shown later that both grids were equally effective in capturing mice for estimates of population and movement. As on the experimental island, the number of traps on the control island did not limit the number of captures in a given trap night.

At the beginning of a trapping period on the islands all traps were opened and baited. The islands were generally trapped concurrently. The traps were tended each morning, rebaited, and left set for the night. This procedure was repeated until the last day of the trapping period when the traps were closed.

The time of tending the traps each day was arrived at by determining the times of capture of mice. On 4 nights in August the traps on the experimental island were run every other hour from 5:00 p.m. to 7:00 a.m. (E.S.T.). When a mouse was caught it was released and the trap reset. A total of 126 captures was made. Mice were most frequently caught between the hours of 7:00 p.m. and 11:00 p.m.; however, 47% of the captures occurred after 11:00 p.m. to 5:00 a.m. Captures at 7:00 p.m. and 7:00 a.m. were virtually zero, as would be expected for a nocturnal mammal. Since mice were captured in numbers over the entire night, traps were not tended until morning.

One obvious disadvantage to this method is that mice caught by 9:00 p.m. (time of peak capture) were held in captivity and perhaps away from their young for 10 to 13 hours—Burt (3) suggests the separation has no deleterious effect. We tried one series of experiments in which a mother and her week-old young were separated for various lengths of time. The experiments were made at air temperature in May in a large bin set up as mouse habitat. Although female and young were apart for as long as 24 hours, the

separation appeared to have no effect upon their survival. These observations and the lack of any data to the contrary from work in the field indicate that the time per se that mice spent in captivity did not cause appreciable harm.

The trapping period required for a good estimate of total population was determined early in the work by sampling known populations. Later, the rate of capture of mice from known population is used as one measure of the efficiency of capture of mice from unknown populations.

In July, 1954, 61 tagged mice were introduced to the experimental island. Trapping began the day of the release and continued for 11 nights. The traps were then closed for 7 nights and opened again for 10 nights. The break in trapping of 7 days was to reduce disturbance. The number of mice caught in each trap night, the "new" captures, and the cumulative percentage of the total introduction caught at least once on successive trap nights are shown in Table I.

TABLE I

Trap night and number of mice captured, number of "new" captures, and cumulative "new" captures as a percentage of 61 mice introduced to the experimental island

| Trap night | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Captures | 21 | 31 | 24 | 31 | 22 | 22 | 28 | 9 | 22 | 18 | 14 |
| "New" captures | 21 | 16 | 4 | 4 | 3 | 1 | 2 | 0 | 0 | 0 | 0 |
| Cum. "new" captures, % | 34 | 61 | 67 | 74 | 79 | 80 | 84 | 84 | 84 | 84 | 84 |

| Trap night | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|---|---|---|---|---|---|---|---|---|---|---|
| Captures | 18 | 19 | 16 | 11 | 11 | 12 | 11 | 9 | 11 | 10 |
| "New" captures | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Cum. "new" captures, % | 85 | 85 | 87 | 87 | 89 | 89 | 89 | 89 | 89 | 89 |

Note in Table I that the total number of mice caught each night dropped relatively slowly while the "new" captures rose quickly until the fourth or fifth night when most of the introduction was caught at least once. By the seventh trap night 84% of the 61-mouse introduction were caught at least once and after 14 more trap nights the capture was increased but 5%. From the results of this experiment, a trapping period of 7 days captured 84% of the mice on the experimental island at least once. This is doubtless a low estimate of trapping effectiveness over the period because death and emigration of mice probably occurred. This would reduce the total number of mice that could be captured and so reduce cumulative "new" captures.

A second introduction of tagged mice to the experimental island in July, 1955, permitted a second test of the effectiveness of trapping method. Twenty tagged mice were released between July 6 and 13. Trapping began on July 26 and in 3 nights of trapping each spaced approximately 1 week apart 18 mice, or 90%, of the introduction were caught at least once. The two mice not caught were not recorded subsequently, hence, the 90% capture is probably a low estimate of trapping efficiency for the 3 nights of trapping.

From these experiments it was concluded that a trapping period of 7 nights provided a near total estimate of mouse population on the experimental island. Hence a census was made over a period of 7 trap nights. Traps were then closed for 7 nights, then opened on the last day to begin another period of census. This basic pattern was altered frequently during the course of the work; sometimes by design, most often by the lack of time to complete a full 7-day period of trapping. The latter condition held in months before May and after August in each year of the study.

*Effectiveness of Trapping Methods*

With the method of census established, consider the effectiveness of the method on both islands over the entire experiment. This is done by (1) comparing rates of new captures from unknown populations with that of a known (introduced) population, (2) comparing the actual catch of mice to the possible catch in a trapping period, and (3) comparing the number of mice caught in one trapping period by live traps to the number caught in a subsequent trapping period by dead traps.

Table II shows the total capture and number of new mice captured in each trap night for three typical trapping periods on the experimental, and control island.

TABLE II

Total capture and capture of new mice by trap night in three trapping periods on the experimental, and control island

| Trapping periods | | Trap night 1 | 2 | 3 | 4 | 5 | 6 | 7 | Totals |
|---|---|---|---|---|---|---|---|---|---|
| Experimental island | | | | | | | | | |
| May 23–29, 1956 | Total captures | 38 | 15 | 16 | 23 | 29 | 23 | 9 | |
| | New captures | 38 | 5 | 1 | 7 | 7 | 1 | 2 | 61 |
| June 6–12, 1956 | Total captures | 34 | 31 | 24 | 24 | 18 | 14 | 11 | |
| | New captures | 34 | 12 | 3 | 2 | 1 | 0 | 1 | 53 |
| June 20–26, 1956 | Total captures | 37 | 29 | 15 | 24 | 6 | 6 | 8 | |
| | New captures | 37 | 5 | 0 | 3 | 0 | 0 | 1 | 46 |
| Control island | | | | | | | | | |
| July 4–10, 1956 | Total captures | 7 | 6 | 9 | 5 | 3 | 2 | 2 | |
| | New captures | 7 | 1 | 3 | 1 | 1 | 0 | 0 | 13 |
| July 12–30, 1955 | Total captures | 18 | 16 | 13 | 24 | 18 | 20 | 19 | |
| | New captures | 18 | 5 | 3 | 1 | 3 | 0 | 0 | 30 |
| Aug. 4–31, 1955 | Total captures | 13 | 17 | 20 | 15 | | | | |
| | New captures | 13 | 6 | 3 | 1 | | | | 23 |

Note in Table II that in each trapping period by approximately the fourth night the number of new captures fell off suggesting that virtually all mice were caught at least once. If the number of individual mice caught in 4 nights of trapping is expressed as a percentage of the total of individual mice caught over an entire trapping period then the rates of capture of new mice can be compared among periods. These are 84%, 96%, and 98% for the experimental island and 92%, 90%, and 96% for the control island. The last figure is calculated for 3 trap nights.

In trapping against a known population (Table I) the rate of capture of "new" mice fell off after approximately 4 nights when 88% of the mice caught in 7 nights were caught at least once. A comparison of the figures indicates that the rates of capture of new mice in the different grids of the experimental and control islands were virtually the same, and these rates are as good as the rate of capture of "new" mice which provided a near total estimate of population of known size.

As a second evaluation of trapping method on each island, trapping efficiency was determined by the method of Hacker and Pearson as described by Snyder (28). In this method the ratio, actual catch of marked mice: possible catch of marked mice is calculated for each trapping period between the first and last trapping period in which mice are captured. The ratio is expressed as a percentage so that a rating of 90% efficient for a trapping period means that 90 out of 100 mice present in a given trapping period were caught.

Table III gives the efficiency of almost all trapping periods in 1954, 1955, and 1956 on the experimental island, and in 1955 and 1956 on the control island. When several trapping periods occurred in a month the data were pooled to give a trapping efficiency for that month. The values shown in italics represent trapping efficiency by months.

When trapping efficiency is compared between the control and experimental islands, the differences in efficiency are not statistically significant (chi-square, $P > 0.05$). This is additional evidence for the conclusion reached above that the two trapping grids were equally effective in capturing mice.

Note in Table III, that when the results of trapping periods are pooled by months in almost all cases, trapping efficiency is increased over the values obtained for the efficiency of trapping periods in the month. Hence, to increase the effectiveness of census, when there were several trapping periods in a month, the data were pooled. Thus, all estimates of abundance were placed on a monthly basis. The month is a natural unit of time in the life of the white-footed mouse besides providing time for an accurate census. A month approximates the time required by a female to produce a litter, and the time required for a major change of pelage in the young.

From Table III, trapping efficiency by months was, or was close to, 100% in most months of the study. Trapping efficiencies less than 100% can be explained by too few trap nights in a month to catch all tagged mice, some mice escaping capture over several trapping periods, or both. In some cases mice that were missed in a month of relatively low trapping efficiency were captured in a subsequent month and could be back-added to the tally to strengthen the census.

The sampling of small mammals by traps may be biased by the heterogeneity of response of the mammals to the traps (Emlen *et al.* (14), Holling (20), Tanaka and Teramura (35), Young *et al.* (39)). If mice became trap-shy as a result of repeated trapping then the disappearance of a mouse could mean a failure of census method rather than an observation of biological significance. To test this condition in the present study, the data on

## TABLE III

Trapping efficiency by trapping period and by month (in italics) on the experimental island (1954 to 1956) and control island (1955 and 1956)

Experimental island, 1954

| Trapping period | July 10–20 | July 27–31 | *July* | Aug. 1–5 | Aug. 11–15 | Aug. 21–23 | Aug. 27–29 | *Aug.* | Oct. 11 | Nov. 15 |
|---|---|---|---|---|---|---|---|---|---|---|
| Trap nights | 11 | 5 | 16 | 5 | 5 | 3 | 3 | 16 | 1 | 1 |
| Actual cap. | 32 | 20 | 26 | 16 | 15 | 12 | 6 | 12 | 4 | 2 |
| Possible cap. | 35 | 27 | 27 | 23 | 16 | 13 | 7 | 12 | 5 | 2 |
| Efficiency, % | 92 | 74 | *96* | 70 | 94 | 93 | 86 | *100* | *80* | *100* |

Experimental island, 1955

| Trapping period | July 26 | Aug. 2 | Aug. 11 | Aug. 25 | *Aug.* | Sept. 2 | Oct. 9 | Nov. 6 |
|---|---|---|---|---|---|---|---|---|
| Trap nights | 1 | 1 | 1 | 1 | 3 | 1 | 1 | 1 |
| Actual cap. | 10 | 11 | 9 | 11 | 12 | 10 | 8 | 3 |
| Possible cap. | 11 | 13 | 12 | 12 | 12 | 11 | 8 | 3 |
| Efficiency, % | *91* | 85 | 75 | 92 | *100* | *91* | *100* | *100* |

Experimental island, 1956

| Trapping period | May 23–29 | June 6–12 | June 20–26 | *June* | July 4–6 | July 18–20 | *July* | Aug. 1–3 | Aug. 15 | *Aug.* |
|---|---|---|---|---|---|---|---|---|---|---|
| Trap nights | 7 | 7 | 7 | 14 | 3 | 3 | 6 | 3 | 1 | 4 |
| Actual cap. | 21 | 38 | 35 | 38 | 29 | 32 | 32 | 22 | 9 | 14 |
| Possible cap. | 25 | 44 | 41 | 39 | 40 | 39 | 37 | 28 | 17 | 16 |
| Efficiency, % | *84* | 87 | 85 | *98* | 73 | 82 | *87* | 79 | 53 | *88* |

Control island, 1955

| Trapping period | June 8–11 | June 14–23 | *June* | July 12–21 | July 28–30 | *July* | Aug. 4–31 |
|---|---|---|---|---|---|---|---|
| Trap nights | 4 | 6 | 10 | 5 | 2 | 7 | 4 |
| Actual cap. | 7 | 9 | 17 | 18 | 16 | 20 | 10 |
| Possible cap. | 9 | 12 | 22 | 18 | 20 | 23 | 13 |
| Efficiency, % | 78 | 75 | *77* | 100 | 80 | *87* | *77* |

Control island, 1956

| Trapping period | May 23–29 | June 6–12 | June 20–26 | *June* | July 4–10 | July 18–24 | *July* | Aug. 1–8 |
|---|---|---|---|---|---|---|---|---|
| Trap nights | 7 | 7 | 7 | 14 | 7 | 7 | 14 | 8 |
| Actual cap. | 10 | 6 | 5 | 5 | 5 | 5 | 4 | 5 |
| Possible cap. | 10 | 9 | 9 | 7 | 7 | 8 | 5 | 5 |
| Efficiency, % | *100* | 67 | 56 | *72* | 72 | 63 | *80* | *100* |

## TABLE IV

Efficiency of trapping mice exposed to one to seven trapping periods

| | Number of trapping periods experienced | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Number of mice | 10 | 5 | 5 | 6 | 12 | 13 | 5 |
| Actual captures | 8 | 9 | 12 | 18 | 47 | 60 | 30 |
| Possible captures | 10 | 10 | 15 | 24 | 60 | 78 | 35 |
| Efficiency, % | 80 | 90 | 80 | 75 | 78 | 77 | 86 |

trapping efficiency were manipulated to compare the efficiency with which mice of different trapping experience were caught.

In Table IV, mice have been grouped according to the number of trapping periods that they have experienced. The periods are from the experimental island from the last week of May to the first week of August, 1956.

Note in Table IV that the efficiency of capture of mice was virtually the same for mice that had experienced one (actually three) and mice that had experienced as many as five (actually seven) trapping periods. We conclude that repeated trapping did not cause an appreciable change in the response of mice to traps. Snyder (28) found the same thing in white-footed mice in Michigan. The conclusion is further strengthened by the third test of trapping.

At the termination of field work, both islands were dead-trapped by placing two dead traps at each set of live traps. The object of this procedure was to catch mice that did not enter live traps. Dead trapping was carried out each night for approximately 2 weeks from the middle of August. Only marked mice were caught.

To summarize trapping methods and the effectiveness of trapping methods on the islands, it is concluded that (1) the trapping methods were not in themselves harmful to mice and did not produce an important change in the response of mice to traps, (2) the 50-ft trapping grid of the experimental island and the 100-ft trapping grid of the control island were equally effective in capturing mice, and (3) the effectiveness of trapping as determined by rate of capture of new mice, ratio of actual: possible capture of mice, and dead trapping was such as to provide in most months figures close or equal to total population.

*Limitation and Errors of Trapping Method*

A limitation of, and several sources of error in, trapping method are noted. This is to prepare for later interpretation of trapping data, and to help explain trapping efficiencies less than 100% recorded above.

A basic limitation of the trapping method of census is that young mice are not tallied until they leave the nest. According to Burt (4), young leave the nest when about 21 days of age. In trapping on the islands and mainland, mice of approximately 21 days of age were frequently caught, while younger mice were rarely captured. These data support Burt's observation and explain the absence of mice younger than approximately 21 days from the population as sampled by traps. In this study, all events in the life of mice younger than approximately 21 days of age must be inferred, or alternatively, direct census data and their analyses are for mice older than approximately 21 days of age. If mice leave the nest at approximately 21 days of age, then allowing a week for first capture, direct census data on young begin at approximately 1 month of age.

In 1954, tunnel traps were used on the experimental island. On several nights mice were heard in traps which, when examined, were empty. Also, mice were found dead with their heads wedged under the doors of traps.

During 2 months of trapping on the experimental island, total capture per trap night fell while set-offs increased from 5% per trap night to 40% per trap night (set-offs are sprung traps without captured animals). On the mainland over the same time, set-offs in tunnel traps were less than 1%. Clearly mice escaped from tunnel traps, and learned to escape with increasing success as they gained experience with traps. The failure of tunnel traps to hold mice probably decreased trapping efficiency and certainly made the analysis of spatial relationships of mice of dubious value. As a result of this experience, the Sherman trap was used in 1955 and 1956. These traps were escape-proof since set-offs, except as the result of other obvious circumstances, were negligible over the rest of the work.

The disturbance of traps affected trapping on the control island in 1956. Over most of May, set-offs were negligible and census efficiency was calculated at 100% (Table III). In the last trapping period in May and on almost every trap night in June, July, and the first 2 weeks in August from 10 to 50% of the traps were set off and often moved short distances from a trapping station. This disturbance of traps explains the relatively low trapping efficiencies calculated for the control island in June and July of 1956. In the second week of August, a long-tailed weasel (*Mustela frenata*) was caught in a live trap on the control island. After this the disturbance of traps ceased. It is likely that the weasel caused all of the disturbance. Considering the kinds of mammals on the study areas, it seems fortunate that more disturbance of traps did not occur.

It was mentioned above that mice were killed by catching their heads under the doors of tunnel traps. Until 1956 the loss of mice to this and other accidents was negligible. In 1956, trapping began in the first week of May of an unusually late spring. In May, 22 mice on the experimental island and 12 mice on the control island died in traps. After May, the number of deaths in traps on both islands was negligible. The accidental removal of mice from the populations reduced the size of sample that was studied for death rate and movement, and doubtless reduced later populations through the loss of offspring. On the other hand, the dead animals provided valuable information that could be obtained only by autopsy.

*Criteria of Age*

The age of animals in a population is an important datum for the calculation of time of birth, birth rate, and death rate. If the ages of animals in a population are known then the dynamics of total population may be related to the contribution of age class to total population.

Age of mice was determined by weight and stage of pelage development. Previous work on the development of *Peromyscus* shows that weight and pelage are reliable indicators of age until adulthood (Collins (11), Dice and Bradley (13), Gottschang (15), McCabe and Blanchard (25)). The age of a mouse is obtained faster by pelage than by weight. In most of the work on the islands, age was determined from the stage of development of pelage of mice.

Several methods were used to convert stage of pelage development into weeks of age for mice on the study area. First, captive mice of known age were studied for growth in weight, and pelage development from 1 to 14 weeks of age and older. Mice of various ages were weighed and described in the conventional manner as juvenile (grey pelage), subadult (grey pelage being replaced by adult pelage), and adult (adult pelage). The data on weight and age were plotted, and a smooth curve of weight on age was drawn for captive mice. Then an estimated weight in grams was read from the curve for mice from 1 to 14 weeks of age and older. The development of pelage with age was followed by expressing the number of mice in a particular pelage in an age group as a percentage of the total number of mice in that age group.

To test the applicability of rates of weight and pelage development of captive mice to the determination of age of mice in nature, mice live-trapped on the control island in 1955 were weighed and checked for pelage development before release. In this case, individual mice were studied over periods of 2 weeks to 3 months. The age of a mouse in weeks was determined at time of first capture by comparison with the weight–age curve drawn for captive mice. The first weight from a mouse was used only to relate the animal to an age in weeks; subsequent weights were used to follow rate of growth. After this manipulation, the rate of growth in weight and the development of pelage with age were calculated for wild mice as for captive mice.

Finally, mice captured on the mainland in dead traps were aged in weeks by referring their weight to the weight–age curve of captive mice. The mainland mice were arranged in groups by weeks of age, and the stage of pelage development of each group was expressed in the same way as for captive mice.

The results of the study of weight and pelage development with age in captive and wild mice are presented in Table V.

TABLE V

Rate of weight and pelage development in captive and wild mice

| | Age, weeks | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14+ |
| **Captive mice** | | | | | | | | | | | | | | |
| Sample size | 3 | 13 | 31 | 34 | 15 | 29 | 6 | 5 | 6 | 5 | 1 | 2 | 6 | 28 |
| Est. wt, g | 4 | 7 | 10 | 12 | 14 | 15 | 16 | 17 | 18 | 18 | 18 | 19 | 19 | 20 |
| Pelage | | | | | | | | | | | | | | |
| % juv. | 100 | 100 | 100 | 89 | 78 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| % subadult | 0 | 0 | 0 | 11 | 22 | 87 | 100 | 100 | 75 | 40 | 0 | 0 | 0 | 0 |
| % adult | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 25 | 60 | 100 | 100 | 100 | 100 |
| **Mice on control island** | | | | | | | | | | | | | | |
| Sample size | | | | | 5 | 6 | 13 | 8 | 10 | 9 | 6 | 9 | 11 | 23 |
| Est. wt, g | | | | | 14 | 15 | 17 | 17 | 17 | 18 | 18 | 19 | 19 | 20 |
| Sample size | | | 2 | 4 | 7 | 11 | 14 | 13 | 11 | 11 | 6 | 11 | 12 | 23 |
| Pelage | | | | | | | | | | | | | | |
| % juv. | | | 100 | 100 | 43 | 18 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| % subadult | | | 0 | 0 | 57 | 82 | 85 | 62 | 64 | 18 | 0 | 18 | 0 | 0 |
| % adult | | | 0 | 0 | 0 | 0 | 15 | 30 | 36 | 82 | 100 | 82 | 100 | 100 |
| **Mice on mainland** | | | | | | | | | | | | | | |
| Sample size | | 2 | 12 | 21 | 23 | 15 | 21 | 22 | | 13 | | 21 | | 46 |
| Pelage | | | | | | | | | | | | | | |
| % juv. | | 100 | 92 | 75 | 57 | 22 | 33 | 9 | | 0 | | 0 | | 0 |
| % subadult | | 0 | 8 | 24 | 30 | 68 | 58 | 68 | | 62 | | 47 | | 0 |
| % adult | | 0 | 0 | 1 | 3 | 0 | 9 | 23 | | 38 | | 53 | | 100 |

Note in Table V that the rates of growth in weight of captive mice and mice on the control island were comparable, which indicates that estimates of age based on rate of growth in weight of captive mice can be applied to wild mice. To relate stage of pelage development to weeks of age the time of change from one stage to another is taken as the week when more than 50% of the sample is in a successive stage of pelage development. In Table V, the short vertical lines in the rows of pelage values indicate the time in weeks that captive, live, and dead-trapped mice were in a particular stage of pelage development. From Table V, the three populations had similar rates of pelage development, which indicates that estimates of age based on rate of pelage development in captive mice can be used on wild mice.

To obtain age in weeks from pelage, the data on the three groups of mice (Table V) were pooled and age in weeks related to stage of pelage development as before. From this synthesis mice in juvenile pelage are from 0 to 5 weeks of age; mice in subadult pelage are from 6 to 9 weeks of age; and mice in adult pelage are 10 weeks of age and older. Our estimates of age based on stage of pelage development are comparable with estimates of age from pelage development of *Peromyscus* in other areas.

## Results and Conclusions

*The Response of Population to Food Supply*

At the beginning of the work in May and June 1953, the experimental island was sampled for white-footed mice by a line of dead traps with 85 sets and two traps at each set. The line was run for 22 nights, the capture of mice was zero.

In July and August a 1-acre grid of dead traps was placed on the control island. The traps were set for 9 nights. A total of three white-footed mice was caught, all in the first night of trapping.

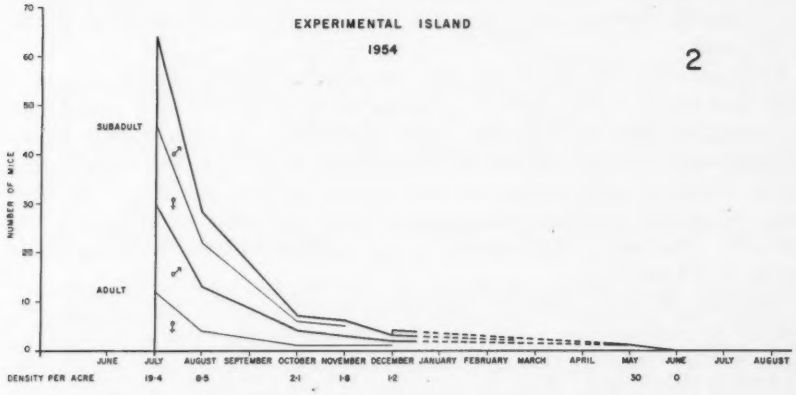
In the same months, two 1-acre grids of dead traps were placed in separate wood lots on the mainland. One quadrat set for 6 nights caught five mice, the other set for 4 nights also yielded five mice. Lines of dead traps set in wood lots yielded the following number of mice per 100 trap nights: June—1, 5; July—2, 1, 0, 1; and August—0, 1, 4.

From the results of trapping both islands and mainland, it was concluded that the experimental island was without a natural population of mice while the control island and mainland supported natural populations of mice in comparable abundance. The density of mice can be estimated from quadrat trapping at three to five per acre, but as Stickel (31) points out, such estimates based on dead trapping likely exaggerate the true density of mice by three or four times.

The absence of mice from the experimental island suggested that either this island offered unsuitable habitat for white-footed mice, or mice could not reach the island, or both. To explore this condition an introduction of mice was made to the island without alteration of the habitat other than the addition of live traps and marker tags.

In July, 1954, 64 mice captured on the mainland were tagged and released on the experimental island. The release was composed of male and female adult and subadult mice in approximately equal numbers. The fate of the introduction was followed by live trapping. The results of the census by months are presented in Fig. 2. Note that by June of 1955 all mice had disappeared from the island. Note also that only one island-born young was recorded in the time of the first introduction: a juvenile mouse captured in December. This seemed odd at the time because several of the females caught in 1954 and 1955 were pregnant as deduced from external appearance.

Throughout the trapping on the experimental island in 1954 and 1955, only introduced mice were captured. Hence, as in the summer of 1953, the island was without a natural population of mice. What is equally important, no immigrant white-footed mice appeared on the island over three summers and two winters to affect population.



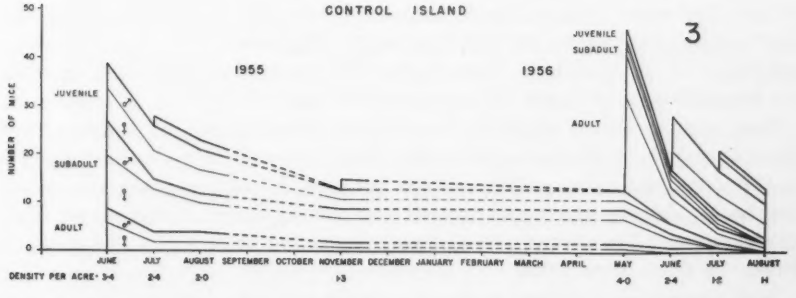




FIG. 2. Number, sex, and age of mice on the experimental island without the addition of food, July, 1954, to June, 1955.

FIG. 3. Number, sex, and age of mice on the control island, June, 1955, to August, 1956.

From May to August of 1954 to 1956, lines of dead traps were run in wood lots of the mainland, and in 1954 on the control island. In 1954 mainland trap lines yielded the following number of mice per 100 trap nights: May—1, 4, 1; June—7, 5, 8; July—12; and August—3, 8. In 1955 the catch was: May—5; June—7, 1; July—11; and August—4 mice per 100 trap nights. From these data mainland populations appeared higher in 1954 and 1955 than in 1953. There is no evidence of a decline in population of mice on the mainland in 1954 or 1955—the population that provided the introduction to the experimental island.

A trap line on the control island in June of 1954 caught eight mice in 100 trap nights. Hence, in 1954 the control island supported a natural population in comparable abundance to the mainland in the same and subsequent year.

From the results of work of 1953 to mid-1955 on the islands and mainland it was concluded that while the island situation may have prevented ingress of mice, the habitat of the experimental island was unsuitable for mice. The next step was to test the hypothesis that the supply of food (or lack of food) was the element of habitat that determined the abundance of white-footed mice.

In June, 1955, 56 food boxes were placed on the experimental island and filled with wheat. The supply of food was maintained in excess of the amount consumed by mice over the rest of the experiment. In July, 1955, a second introduction of 20 mice from the mainland was made to the experimental island. The second introduction consisted of juvenile, subadult, and adult mice in approximately equal numbers. The sex ratio of the group was 100:100. The fate of the introduction was followed by live trapping over a period of 14 months.

Beginning in June, 1955, the population of mice on the control island was studied by live trapping in the same manner as on the experimental island. Results of live trapping the experimental and control islands over the summer and fall of 1955 and the spring and summer of 1956 are shown in Figs. 3 and 4.

Note in Fig. 3 that densities of mice on the control island ran from 1.1 to 4.0 mice per acre. Lines of dead traps on the mainland in 1956 yielded the following number of mice per 100 trap nights: May—0, 4, 3, 2; June—1, 3, 1; and July —3, 0, 1. These data suggest that mainland population in 1956 was lower than in 1954 and 1955, as recorded above.

Note now in Fig. 4 the densities of mice recorded on the experimental island from July, 1955, to August, 1956, under the condition of food in excess. In this time, densities of mice ranged between 3.9 and 34.2 mice per acre! The lowest density represents the mice of the second introduction by September prior to the appearance of their offspring in the traps. The greatest density is from May, 1956.

With the addition of food to the experimental island in 1955 we maintained a population of mice where none existed in 1953, and where none would exist when introduced to the island in 1954. Moreover, the densities of mice produced from the introduction in 1955 were greater than the densities of

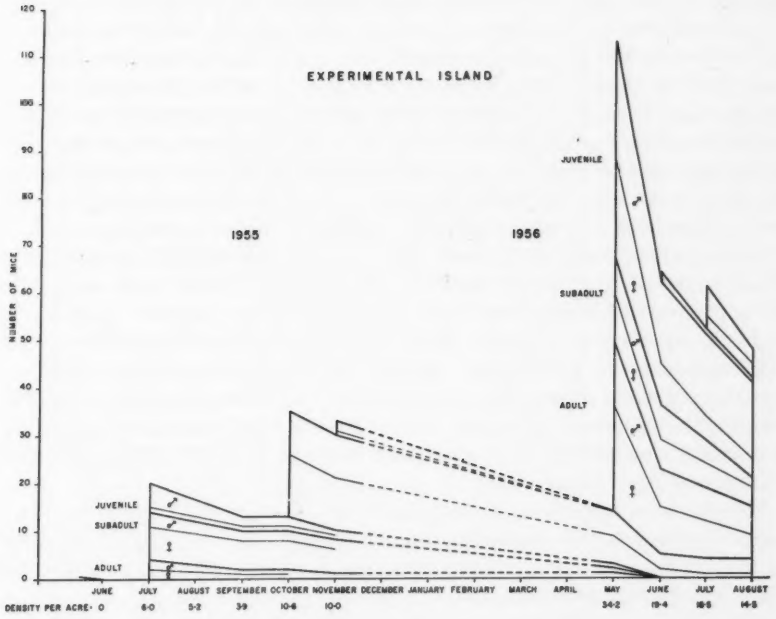



FIG. 4. Number, sex, and age of mice on the experimental island with the addition of food, July, 1955, to August, 1956.

mice recorded in 1955 and 1956 on the control island, which did not receive the experimental treatment. From mainland dead trapping, the response occurred on the experimental island when mainland populations were relatively low. The general conclusion from these results is that the supply of food is a factor that limits the abundance of white-footed mice in nature.

*The Effect of Food Supply on Population: Dispersal*

The factors that determine population are birth rate, death rate, and dispersal. Consider now how food supply affects population of mice from an evaluation of these factors on the experimental and control islands.

The change in numbers of mice on the two islands could be explained by dispersal or the movement of mice to and from the islands. The islands were selected as study areas because of the water barrier that separated them from each other and the mainland. From December to March the barrier is in the form of ice and snow. The island situation was to prevent dispersal, and so remove one factor affecting population.

There are data to show that the water barrier prevented movement of *Peromyscus* to the experimental and control islands. In 1953, 1954, and 3 months of 1955, mice of the first and second plantings were the only mice recorded on the experimental island. Then when most untagged mice

appeared in the traps of both islands they did so as groups of young mice related to the breeding activity of resident, tagged adults. Once the untagged animals on the islands were marked in a week or two of trapping, new individuals did not appear in the populations until after a time of breeding activity. Finally, from Figs. 3 and 4, the greatest difference between densities of mice on the islands can be attributed to a greater number of young mice appearing in the traps in May, and later, on the experimental island. As noted above, the young mice were doubtless island-born young. We conclude that immigration was unimportant as a factor affecting the island populations.

On the other hand, mice and other small mammals did cross between islands and mainland so the island situations were not exclusive. In May, 1956, a group of untagged adult mice appeared in the traps on both islands (Figs. 3, 4). These animals were immigrants and/or island-born young which appeared on the islands between fall and spring trapping. With the conclusion reached above it is assumed that the untagged adults were mice born on the islands. The point is relatively unimportant; however, a later conclusion is qualified by the possibility that the untagged adults originated all or in part by immigration.

Movement of mice from the islands is difficult to evaluate because when a mouse disappears from the traps it may have died on the island or emigrated. In this study, death and emigration of mice are treated as one statistic for both act to decrease population.

There is direct evidence that emigration from the islands did occur. Five mice tagged and released on the experimental islands were subsequently trapped on the mainland. One was an adult female placed on the experimental island in July, 1955. She was trapped on the mainland shore in the same month. The mouse was returned to the island where she stayed as indicated by later captures. One mouse was recorded on the control island on July 19. On July 21 of the same year it was captured on the mainland approximately one-third of a mile from the original point of capture. As might be expected, four of the five emigrants were last recorded as juvenile or subadult mice.

*Death Rate of Mice Captured in Live Traps*

Death rate or survival rate is calculated from the number of tagged mice that disappear from the traps in each month. This seems an accurate calculation, for once a mouse was missed in a census it was rarely recorded again. The disappearance of mice from the islands can be followed in Figs. 2, 3, and 4. Note in the three figures that there was a decline in number of mice of each age group and sex from the month of initial capture.

As a first step in the analysis of death rates on the experimental and control islands, the rate of loss of males and females of each age class was tested for difference by chi-square $R \times C$ test. This test and the 5% level of probability were used throughout the analyses of rates of disappearance of mice of different sex, age, and time of birth. In these analyses the number of mice killed in trapping were excluded unless negligible so that deaths were the result of natural events. As might be concluded from inspection of Figs. 2,

3, and 4 there is no statistically significant difference in the disappearance by months of male and female mice in each age class. Hence male and female mice had the same death rate. With this conclusion the data on disappearance of males and females were pooled to give disappearance of mice.

Data on the disappearance of mice of different age groups, as read from Figs. 2, 3, and 4, were analyzed to compare the death rates of mice of different ages and born at different times of the year on the experimental and control islands. Data for this analysis are presented in Table VI. In the analysis, the rates of disappearance of the subadult and adult mice of the 1954 introduction were tested for difference. This was also done for all age classes of mice on the experimental island in 1955 and 1956 and on the control island in the same years. Despite the fact that the mice were of different age groups and began life at different times of the year, there was no statistically significant difference in death rate among the age groups of mice. Hence, juvenile, subadult, and adult mice taken in live traps had the same death rate and this rate was independent of the time of appearance of an age class in the traps. This result also suggests that death rate is independent of age.

With this conclusion, the age groups in the three sets of data were pooled and started together at month one to compare death rates of mice of the first introduction, mice of the second introduction, and mice of the control island. There was no statistically significant difference between the death rates of mice on the experimental and control islands from 1955 to 1956. On the other hand, the death rate of mice of the first introduction to the experimental island in 1954 was significantly greater than the death rate of all other mice.

The best estimates of death rate of mice of the first introduction, and of all other mice, were then calculated from the pooled data on the disappearance of mice from the experimental island in 1954, and the pooled data on the disappearance of mice from the two islands in 1955 and 1956. The pooling of data from the experimental and control islands in 1955 and 1956 was complicated by gaps in census data over the life span of some groups of mice, and census data short of the life span of others. Both conditions act to change from month to month the size of sample of mice in which deaths are followed. To bring the survivors of the pooled sample to a common base, the survivors in each month were expressed as a percentage of their original number in month one. This calculation is given in the last column of Table VI for mice of the two islands from 1955 to 1956. A similar calculation for mice on the experimental island in 1954 is given in the fourth column of the data of 1954.

It was anticipated from the way that mice disappeared in the two sets of data that death rates were constant. To test this conjecture and also to calculate rates of death, the number of mice in per cent surviving in each month on the experimental island in 1954, and on the experimental and control islands in 1955 and 1956, were converted to logarithms and plotted against months from month one. A best straight line was fitted to each set of data by the method of least squares. The results of this procedure are shown in

TABLE VI

Number of mice of different age groups alive on the experimental and control islands by months after month of first capture

| | Experimental island | | | | | | | | | | | | | Control island | | | | | Experimental and control islands 1955 to 1956 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1954 | | | | 1955 | | | | 1956 | | | | | 1955 | | | 1956 | | | |
| Month | Subad. | Ad. | Total | % | Juv. | Subad. | Ad. | Juv. | Juv. | Subad. | Ad. | | | Juv. | Subad. | Ad. | Juv. | Ad. | Total | % |
| 1 | July 30 | 29 | 59 | 100.0 | July 6 | 10 | 4 | Oct. 22 | May 44 | 16 | 23 | | | June 12 | 18 | 8 | June 11 | May 17 | 191/191 | 100.0 |
| 2 | 15 | 13 | 28 | 47.5 | 5 | 9 | 3 | 20 | 26 | 13 | 18 | | | 11 | 11 | 4 | 9 | 7 | 136/191 | 71.2 |
| 3 | — | — | — | — | 3 | 8 | 2 | — | 22 | 10 | 15 | | | 9 | 8 | 4 | 7 | 3 | 91/169 | 53.9 |
| 4 | 4 | 3 | 7 | 12.5 | 3 | 8 | 2 | — | 20 | 6 | 11 | | | — | — | — | — | 2 | 52/120 | 43.3 |
| 5 | 3 | 3 | 6 | 10.7 | 2 | 7 | 1 | — | — | — | — | | | — | — | — | — | — | 10/20 | 50.0 |
| 6 | 1 | 2 | 3 | 5.4 | — | — | — | — | — | — | — | | | 4 | 7 | 2 | — | — | 13/38 | 34.2 |
| 7 | — | — | — | — | — | — | — | — | — | — | — | | | — | — | — | — | — | — | — |
| 8 | — | — | — | — | — | — | — | 11 | — | — | — | | | — | — | — | — | — | 11/22 | 50.0 |
| 9 | — | — | — | — | — | — | — | 5 | — | — | — | | | — | — | — | — | — | 5/22 | 22.7 |
| 10 | — | — | — | — | — | — | — | 4 | — | — | — | | | — | — | — | — | — | 4/22 | 18.2 |
| 11 | 0 | 1 | 1 | 1.8 | 1 | 1 | 1 | 4 | — | — | — | | | — | — | — | — | — | 7/42 | 16.7 |
| 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | — | — | — | — | | | 4 | 7 | 2 | — | — | 13/58 | 22.4 |
| 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | — | — | — | — | | | 2 | 3 | 1 | — | — | 6/58 | 10.3 |
| 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | — | — | — | — | | | 1 | 0 | 1 | — | — | 2/58 | 3.5 |
| 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | — | — | — | — | | | 0 | 0 | 0 | — | — | 0/58 | 0.0 |

— No census data.

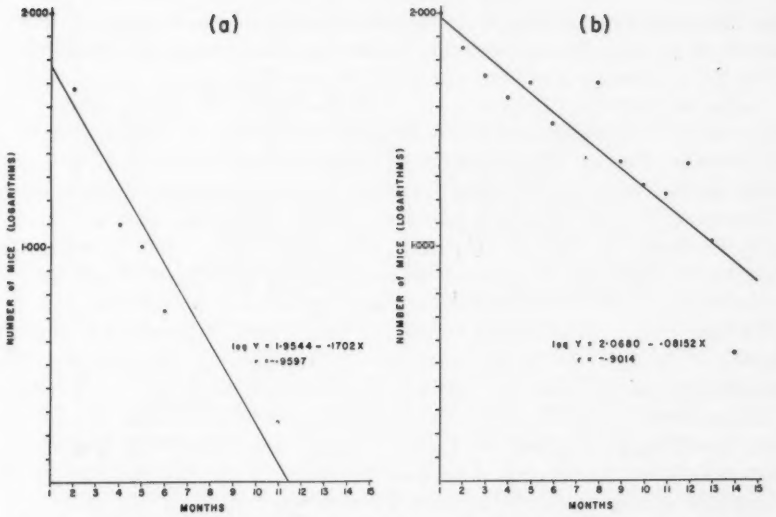



FIG. 5. (*a*) Death rate of mice on the experimental island without food in excess. (*b*) Death rate of mice on the experimental island with food in excess and on the control island.

Fig. 5(*a*) for the experimental island in 1954, and Fig. 5(*b*) for both islands in 1955 and 1956. The correlation coefficient "*r*" is given for each line, and in both cases the value is statistically significant at the 5% level for a linear correlation. Hence, death rate of mice captured in live traps, or from approximately 1 month of age, is constant and independent of age.

The death rates of the two groups of mice are obtained from the slopes of the regression lines. In mice of the first introduction to the experimental island the death rate is log 0,1702 mice per month. To convert this into ordinary numbers multiply log 10 by $\log_e 10$ or 2.30259. From this calculation the death rate of mice introduced to the experimental island in 1954 when food was not supplied was 0.3919, or approximately 39% per month. A similar calculation for the death rate of mice on both islands from 1955 to 1956 and when food was supplied to the experimental island gave a death rate of 0.1877, or approximately 19% per month; almost half as great as the death rate of mice of the first introduction.

When food was added to the experimental island the death rate of mice from approximately 1 month of age was decreased. This decreased death rate was not significantly different from the death rate calculated for a natural population of mice. The conclusion from this observation is that food supply, below some low level, affects the death rate of white-footed mice of approximately 1 month of age and older. Above this low level, food supply has no effect on death rate from approximately 1 month of age.

The meaning of death rate is qualified by two conditions. As noted before, mice could not be censused until they left the nest and came to traps. Hence,

the above observations on death rate apply only to mice of approximately 1 month of age and older. Secondly, death rate is a combination of emigration from the islands, as mentioned above, and mortality on the islands.

There are few data from this study on the causes of natural mortality of mice, or on the importance of death over emigration as a cause of the disappearance of mice. Since most mice were repeatedly captured on a home range on the islands it is difficult to believe that most disappearance represents emigration. Other studies have shown that *P. leucopus* does not have a great tendency to disperse (Burt (3), Nicholson (26)). Then Snyder (28) calculated constant death rates for white-footed mice in Michigan that are close to the death rates (death and dispersal) of mice on the two islands in 1955 and 1956. This suggests that our death rate is mostly the result of deaths. Finally, the island situation lends weight to the argument that emigration was relatively unimportant as a factor in death rate on the islands.

What would cause the death of mice on the islands is almost entirely unknown. The most reasonable explanation at the present time is predation. The remains of two tagged mice were found on the control island near live traps and it is presumed that both animals were killed by a weasel. It is worthy of note that despite the presence of a weasel on the control island over part of the summer of 1956, the death rate on the island was the same as the death rate of mice on the experimental island over the same time.

On the other hand, an explanation of death rate of mice must take into account the observation that when food was not supplied to the experimental island, death rate was twice as great as when food was supplied in excess. It would appear that with a low level of food supply, predation or emigration or both increased. Strecker (33) observed when house mice (*Mus musculus*) were short of food, emigration occurred. Hence, emigration may have been an important factor in the disappearance of mice from the experimental island when food was not supplied in excess.

To conclude, it is not possible to resolve death rate as calculated here, for mice of approximately 1 month of age and older, into actual mortality on the islands, and migration from the islands. However, both factors act to decrease population.

When food was not supplied in excess to mice of the first introduction, death rate or the rate of disappearance of mice from the traps greatly exceeded the appearance of new individuals. Hence, the first introduction failed. After food was supplied in excess to the experimental island, the death rate of live-trapped mice of the second introduction was comparable to the death rate of live-trapped mice on the control island. At the same time, the population of mice on the experimental island increased to densities from 9 to 15 times as great as densities of mice on the control island (Figs. 3 and 4). Note in Figs. 3 and 4 that the greatest difference between islands in density of mice was from the greater number of young mice on the experimental island in 1956. Death rates of mice from 1 month of age were the same on both islands and immigration was unimportant as a factor affecting population.

Clearly then, a second and major effect of food supply on population is to be found in an increased birth rate, or a decreased death rate of young, or both.

*Birth Rate and Death Rate of Young*

The combined birth and death rates of young mice on the islands is measured as the ratio of adult female mice to offspring captured in traps. From Fig. 2, when food was not supplied in excess, 13 adult females in August had no descendants by the following May. From Fig. 4, when food was supplied in excess in 1955, 8 adult females in August had 46 adult descendants by the following May, approximately 6 per female. On the control island in the same year and period (Fig. 3), 14 adult females had 27 adult descendants, or approximately 2 per female. This calculation assumes that the untagged adult mice on the islands in May were island-born. The difference between the experimental and control islands in 1955 and 1956 in descendants per adult female is statistically significant (chi-square $2\times2$ table, $P < 0.05$).

Consider the number of juvenile and subadult mice per adult female trapped on both islands in the spring of 1956 (Fig. 3, 4). On the control island in May, there were 27 adult females to 6 young, or 0.22 young per female. On the experimental island in the same month there were 31 adult females to 64 young, or 2.1 young per female; approximately 10 times as many young per adult female as the control island! It was not until June, or approximately 1 month later, that the bulk of the spring-born young appeared in the traps of the control island. By June on the control island, the ratio, adult females: spring-born young, among trapped animals, was 9:17, or 1 female to approximately 2 young. On the experimental island over the same period, the ratio was 12:66, or 1 female to approximately 6 young, a threefold greater production of young of approximately 1 month of age and older. The difference is statistically significant (chi-square $2\times2$ table, $P < 0.05$).

The conclusions from the analysis of birth and death rate of young by ratio of adult females to descendants are (1) below some low level of food supply the production of mice to approximately 1 month of age virtually ceases, and (2) food in excess results in an increase in the number of offspring per adult female taken in traps. These conclusions explain in most part the greater density of mice on the experimental island with food in excess as compared with the same island when food was not added, and the control island.

*The Effect of Food Supply on the Production of Young: Breeding Biology*

The next step in the determination of the effect of food supply on population is to show how food supply increases the number of offspring per adult female taken in traps. The ratio could be affected here by a change in (a) litter size, (b) frequency of litters, (c) the number of young raised to approximately 1 month of age, (d) a combination of all three factors.

The breeding biology of *P. l. noveboracensis* has been described by several authors. Coventry (12) found that the breeding season extended from April to October, and from counts of young *in utero* in 50 females, mean litter size

was $5.04 \pm 0.08$. Burt (3) places the beginning of the mating season in March with the first young born in late March and April. Males come into breeding condition at the same time as females. Burt describes a rest period of a month or more between spring and fall breeding for mature females. The last litters appear in September and early October. A mature female that lives throughout the breeding season has two or three litters in the spring and two in the fall. Young females of the year begin to breed at $2\frac{1}{2}$ or 3 months of age, although apparently none of the young born in August onward breed until the following spring. Mean litter size at birth in Burt's animals was 4.26 from 39 females. Svihla (34) calculated a mean litter size at birth as $4.36 \pm 0.10$ for 53 litters of *P. l. noveboracensis* born in captivity.

In the present study, breeding biology was determined from the appearance and weight of the reproductive organs of mice trapped in the field, and from the birth of mice in the colony. The age class composition of the island and mainland populations was also a clue to breeding activity.

Data on the reproductive condition of white-footed mice captured on the mainland of the study area in 8 months of the year are presented in Table VII. Data from 1954 to 1956 are combined.

Note in Table VII that February and December were months of sexual dormancy with the reproductive organs of adult males at their smallest size and none of the females pregnant. The presence of corpora lutea in a few of the females suggests a sterile ovarian cycle in winter months.

TABLE VII

Month and reproductive condition of mice on mainland

| Month | Age | Female sample | No. with old set of corpora lutea | No. with fresh set of corpora lutea | No. pregnant | Male sample | Wt. male rep. organs* |
|---|---|---|---|---|---|---|---|
| Feb. | Ad. | 3 | 0 | 1 | 0 | 1 | 0.2, – |
| March | Ad. | 2 | 0 | 0 | 0 | 5 | 0.4, 0.3–0.7 |
| April | Ad. | 11 | 4 | 8 | 8 | 12 | 0.9, 0.5–1.2 |
| | Subad. | – | – | – | – | – | – – |
| | Juv. | 1 | 0 | 0 | 0 | – | – – |
| May | Ad. | 5 | 4 | 5 | 4 | 13 | 0.9, 0.4–1.4 |
| | Subad. | – | – | – | – | – | – – |
| | Juv. | 5 | 0 | 1 | 1 | – | – – |
| June | Ad. | 7 | 6 | 5 | 2 | 13 | 0.7, 0.2–1.3 |
| | Subad. | 10 | 1 | 2 | 0 | 13 | 0.2, 0.1–0.4 |
| | Juv. | 9 | 0 | 2 | 0 | 12 | 0.1, <0.1–0.3 |
| July | Ad. | 6 | 6 | 6 | 5 | 9 | 1.0, 0.1–1.5 |
| | Subad. | 10 | 1 | 2 | 1 | 32 | 0.3, 0.1–0.7 |
| | Juv. | 6 | 0 | 2 | 0 | 8 | 0.2, <0.1–0.7 |
| Aug. | Ad. | 6 | 2 | 4 | 3 | 5 | 1.0, 0.5–1.4 |
| | Subad. | 4 | 1 | 4 | 0 | 9 | 0.6, 0.2–1.0 |
| | Juv. | 2 | 0 | 0 | 0 | 2 | 0.2, 0.2–0.3 |
| Dec. | Ad. | 22 | 4 | 4 | 0 | 11 | 0.1, <0.1–0.2 |

*Mean and range.

Sexual activity begins in March as indicated by the increased size and weight of the reproductive organs of the adult males. Pregnant adult females were found in all months from April to August. Adult females captured in April had two sets of corpora lutea, which indicates two pregnancies and at least one litter by April.

In the period April to August, 1 of 23 juvenile females and 1 of 24 subadult females were pregnant, as determined by embryos *in utero* visible to the naked eye. In the same time, 22 of 35 adult females captured were pregnant. Hence, most females do not breed until they are in adult pelage or 10 weeks of age and older. The small size and weight of the reproductive apparatus of juvenile and subadult males suggest that, like females, they do not begin to breed until adult pelage is obtained.

Litter size was determined for mice on the mainland from counts of viable embryos *in utero*, and for mice in the laboratory colony from counts of nestlings at time of birth. Mean litter size in 23 females trapped on the mainland was $5.52 \pm 0.20$. Mean litter size in 82 litters born in the laboratory was $4.22 \pm 0.15$. The difference between mainland and laboratory mice in size of litter is statistically significant ($t$-test, $P < .05$). The explanation is not known. In the laboratory several litters came from the same female so that age of mother might have been a factor determining the relatively small size of litter. When litters from the laboratory were grouped according to pregnancy (hence age of mother) there was no statistically significant difference in litter size among first to sixth pregnancy (simple analysis of variance; $F = 2.53$, $F$ at $P = 0.05$ is 2.54). One possible explanation for the relatively small average size of litter is that in captivity, mothers frequently ate some or all of their young at birth.

Upon this background of breeding biology, consider the reproductive activity of mice on the islands to find how food supply affects the ratio, adult females: offspring. Factors to be considered are litter size, frequency of litters, and the survival of young from birth to approximately 1 month of age.

*The Size of Litters on the Islands*

The size of litters from adult females on the two islands was estimated by counts of viable embryos in 17 pregnant adults taken from the experimental island in May and August of 1956, and 10 pregnant adults trapped on the control island in the same time. Mean litter size on the island with food in excess was $5.17 \pm 0.29$, and on the control island $5.20 \pm 0.36$. The differences in litter size among the experimental and control islands and the mainland ($5.52 \pm 0.20$) are not statistically significant (simple analysis of variance; $F = 0.636$, $F$ at $P = 0.05$ is 3.19). Hence, food in excess did not affect the size of litters of mice on the experimental island. Moreover, increased size of litter does not explain the difference between the two islands in number of young mice per adult female.

*The Frequency of Litters on the Islands*

The frequency of litters produced by a female mouse is most likely determined by the length of the breeding season, and the age of first breeding and later, fecundity of the female.

The length of breeding season of mice on the islands and mainland was measured from animals trapped throughout the year. In the last week of February of 1957, five adult males were trapped on the experimental island, and five adult males and four adult females were trapped on the control island. The means and ranges of weights in grams of the reproductive organs of the males were 0.5, 0.3–0.7 for the experimental island and 0.4, 0.2–0.5 for the control island. The reproductive organs of the females were inactive. Three adult males were caught on the control island in April of 1957. At this time their reproductive organs averaged 1.1 g with a range of 1.0 to 1.1 g. From these data, reproductive activity of mice in February and April on the islands was comparable to that of mice on the mainland in the same time (Table VII).

From Figs. 3, 4, and 5, juvenile mice born on the islands appeared in the traps in May, June, July, October, November, and December. Since juveniles that were trapped are 3 to 5 weeks of age, then females were pregnant in April, May, June, September, October, and November. Subadult mice appeared in the traps in May on the two islands. If subadults are 6 to 9 weeks of age, then females were pregnant in March and April. Autopsy of mice from the islands and mainland showed that females were pregnant in each month from April to August (Table VII and below).

The data on time of breeding activity suggests that mice on the islands and the mainland had the same length of breeding season; from March to November, and this coincides with the breeding season that is recorded in the literature for the white-footed mouse. Hence, it is concluded that food in excess did not lengthen the breeding season of mice to increase the frequency of litters.

There is some evidence to suggest that food in excess affects the age of first breeding in the female. It was noted above that eight females on the experimental island in August of 1955 had 46 adult descendants by May of 1956. Eight females dying at the rate of 19% per month and producing five young per female per month subject to the same death rate could not have produced 46 adult descendants by May. If it is assumed that none of the 46 mice were immigrants, then females born in the fall on the experimental island must have become members of the breeding population in the fall of their birth. On the control island, with the same assumption, the number of adult descendants in May, 1956, from adult females in August, 1955, can be explained by at least two fall litters from each female, without the contribution of young born in the fall to the breeding population. This explanation would be in keeping with Burt's description of fall breeding activity of white-footed mice in Michigan as noted above.

On the other hand, fall-born females may have bred on the control island, but death of offspring reduced their contribution to the spring population. With the lack of conclusive data at the present time, it is stated that food in excess may result in fall-born females breeding in their first fall so that the frequency of litters is increased. If this is true, then food in excess increased the birth rate of mice on the experimental island. However, such an increased birth rate does not explain the great difference between islands in young per adult female in the spring of 1956. Hence, the factors: litter size, length of breeding season, and age of first breeding are eliminated as important causes of the density of mice on the experimental island. Clearly the effect of food in excess is on the fecundity of females, the survival of young, or both.

*The Fecundity of Females and the Survival of Young on the Islands*

In May of 1956, 15 adult females from the experimental island and 9 adult females from the control island were autopsied. Seven of the 15 and 5 of the 9 were pregnant in May. Four of the 5 adult females collected on the mainland in May were pregnant (Table VII). These data suggest that island and mainland adult females were equally fecund. This conclusion is supported by the observation that 14 of the 15 and 9 of the 9 females had sets of recent corpora lutea. If most corpora lutea in the white-footed mouse represent corpora lutea of pregnancy (Ironside (21)), then nearly all the adult females on both islands were pregnant in or before May. To carry the argument further, 9 of the 15, and 8 of the 9 females had sets of old or resorbing corpora lutea, which indicate at least two pregnancies in these females by May. The data from island females are comparable with those from the mainland (Table VII).

If the adult females on the islands in the spring of 1956 were equally fecund, this leads to but one conclusion. The difference between the two islands in abundance of mice is the result of a difference in death rate of young. The sets of old corpora lutea represent the litters of mice that were born to almost all adult females on the islands in March and April. On the experimental island the litters appeared in the traps in May as juveniles and subadults. On the control island, however, a higher mortality of young occurred so that relatively few young appeared in the traps.

The death rate of young between time of birth and approximately 1 month of age can be compared between islands by calculating for each island the number of young born (as given by sets of corpora lutea and litter size) to number of young in the traps. This calculation assumes no emigration of young and takes 5.2 as the average size of litter at birth. By May, on both islands, most of the females had two sets of corpora lutea and therefore had produced at least one litter. The number of young at birth on the experimental island in May is estimated as the number of adult females in May (31) times average size of litter (5.2), or 161 young. Of these 161 young, 64 appeared in the traps; hence death rate was 97/161, or 60%. A similar calculation for the control island yields a death rate of 134/140, or 96%.

The relatively low death rate of young on the experimental island explains in most part the difference between islands in the density of mice. Moreover, a high death rate of young explains, most satisfactorily, the virtual absence of young mice from the traps of the experimental island after the first introduction was made.

The conclusion from the above analysis is that death rate of young mice was a major factor affecting the abundance of white-footed mice on the two islands. When food in excess was supplied to the experimental island the death rate of young decreased and population increased to greatly exceed the densities of mice on the same island before food was supplied in excess, and on the control island. Hence, the supply of food determines population mainly by affecting the survival of young between birth and approximately 1 month of age.

## The Limitation of Population on the Experimental Island

Since the supply of food did not limit the population of mice on the experimental island from July, 1955, on, how was population limited? This question is better asked as how would population be limited for there is no reason to believe that the experimental population had reached an upper limit of density under the condition of food in excess. Note in Figs. 4 and 5 that populations on both islands began to decline after peak densities of May. The decline was the result of death and emigration, and the summer check in breeding which is apparently independent of the supply of food. It is likely that with the fall production of young the density of mice on the experimental island would have been greater than 34.2 to the acre, the density noted in spring. Hence, a search for limiting factors, other than the supply of food, is a search for incipient limiting factors if any were present at all at this stage of population growth.

Three density-dependent phenomena were examined as possible limiting factors to the population of mice on the experimental island. The phenomena were (1) disease, (2) stress as a result of exhaustion of the pituitary–adrenocortical system as reflected by trap mortality, stunted growth, and enlarged adrenal glands, and (3) intraspecific strife for space as measured by the movements of mice between traps.

### *Disease as a Limiting Factor*

Infectious disease may be regarded as a factor that can limit population (Bendell (1)), or a factor to which certain animals are less resistant at some population densities than at others (Chitty (6)). In either case a difference in kind and frequency of disease between dense population on the experimental island, and relatively sparse population as on the control island and mainland, would suggest a difference in welfare between populations.

In 1956, adult mice trapped on the mainland and the two islands were examined for disease. In the course of autopsy, sections of the liver and the contents of the gut of each animal were examined under a 10× dissecting

microscope. The mice from the islands were taken in May and August while the mainland sample was obtained from May to August. The kind and frequency of parasitic infection found are presented in Table VIII.

TABLE VIII

Kind and frequency of parasitic infection in mice captured on the mainland and islands, 1956

| Parasite | | Size of sample | | |
|---|---|---|---|---|
| | | Mainland, 24 | Control island, 14 | Exptl. island, 46 |
| *Reticularia coloradensis* | (Nematoda) | 7 | 0 | 0 |
| Physalopteridae | (Nematoda) | 1 | 0 | 0 |
| *Brachylaemus peromysci* | (Trematoda) | 4 | 0 | 6 |
| *Taenia mustelae** | (Cestoda) | 1 | 0 | 0 |
| *Cuterebra* sp. | (Diptera) | 1 | 0 | 2 |
| Mite | (Acarina) | 0 | 1 | 0 |
| *Ixodes augustus* | (Acarina) | 1 | 0 | 0 |
| *Orchopeas leucopus* | (Siphonaptera) | + | + | + |

*Cysticerci.
+Present.

From Table VIII, the difference between experimental island, control island, and mainland mice, or dense and relatively sparse population in kind and frequency of parasites was not striking. Moreover, serious debilitation to the host was not associated with any parasitic infection. In addition to these relatively detailed autopsies, over 3 years of study, more than 200 mice from the mainland and islands were superficially examined. In no case was a debilitating disease observed. These data do not mean that all diseases were detected or that disease might not have become important as a population control. From the available data it is concluded that disease was unimportant as a factor affecting the relatively dense population on the experimental island.

*Stress as a Limiting Factor*

Christian (7) postulated that stress or exhaustion of the pituitary–adrenocortical system by stressors inherent in high population could cause decreased birth rates and increased death rates to decrease population. Some stressor conditions are fighting, severe climatic conditions, and the physiological demands of the spring breeding season. In a series of experiments with captive and wild rodents Christian (*in* Christian and Davis (9)) has demonstrated a density-dependent stimulus to the pituitary–adrenocortical system which is independent of food, water, and shelter. Symptoms of pituitary–adrenocortical stimulation are an enlargement of the adrenal cortex, decreased weight of the reproductive organs, and decreased reproduction. It is presumed that stressed animals are susceptible to additional insult from within or without. Christian and Davis (8) consider that stunting of growth is a reflection of increased stress in a population.

Two methods were used to evaluate the condition of stress in adult mice in dense population on the experimental island, and relatively sparse population on the control island and mainland. First, mortality in live traps in dense and sparse populations on the islands was compared. A high trap mortality per capture in dense population would suggest that capture was an insult that further stimulated animals already stressed by stressors inherent in high population to the complete exhaustion of the pituitary–adrenocortical system and the death of the animal.

Nearly all deaths in live traps occurred in the first and second week of May, 1956. May is part of the spring breeding season and in 1956 was the time of peak population on both islands as well as part of an unusually cold spring. The density of mice on the experimental island was 34.2 to the acre; approximately 9 times the density of mice on the control island (Figs. 3, 4). Hence, conditions appeared favorable for the development of stress in the more dense population.

Over the period May 4 to 9, 1 male in 36 captures and 10 females in 57 captures died in live traps in dense population. In the same time, 2 males in 20 captures and 10 females in 42 captures died in live traps in sparse population. Significantly more adult females died in traps than adult males, but there is no statistically significant difference between dense and sparse populations in number of adult males and females dying in traps (chi-square, $2\times2$ table, $P > 0.05$). Hence, density of population was not correlated with loss of animals in traps. From this there is no indication that animals in dense population were more highly stressed than those in sparse. Since almost all deaths occurred in cold weather it appears that exposure to cold was the cause of death.

The second approach to an evaluation of a physiological difference in mice in different densities of population was to compare body size, and weight of paired adrenal glands (as a measure of adrenal cortex) of adult mice of both sexes in dense and relatively sparse populations. The samples of mice compared were 18 males and 25 females from the experimental island, 4 males and 12 females from the control island, and 25 males and 12 females from the mainland. All were collected from May to August in 1956. In addition, 12 males collected on the mainland from May to August in 1955 were included in the comparison. The groups of mice were tested for difference in body size (length of head and body), and weight of paired adrenal glands, by simple analysis of variance. There was no statistically significant difference among populations in body size or weights of paired adrenal glands in either sex (for body size, $F=1.36$, $F$ at $P$ $0.05=2.76$; for adrenal weights, $F=0.799$, $F$ at $P$ $0.05=2.76$). From this and the above result the symptoms of stress, increased death rate as a result of additional stimulation from the environment (trapping), stunting of body size, and hypertrophy of the adrenal glands, were not present in dense population as compared with sparse. Hence, it is concluded that pituitary–adrenocortical exhaustion was unimportant as a factor affecting densities of mice on the experimental island.

*Intraspecific Strife for Space as a Limiting Factor*

The third density-dependent phenomenon to be investigated as a possible limiting factor to population on the experimental island was intraspecific strife for space. In this analysis, space is measured as the distance animals move between traps. Strife is measured by the distribution of mice in dense population, and the decreased distances that animals move between traps with increased density of population. The movements of mice were studied by plotting points of capture, and by measuring the distances animals moved from their homes or the trapping stations where they were most frequently caught. In most cases a home was the trapping station near a hole or holes in rock and forest litter into which released mice were observed to plunge. The latter method of analysis is like the measurement of movement from a center of activity described by Hayne (17).

Figure 6 shows the homes and ranges of male and female mice in dense population. Frequency of capture of mice ranged from 2 to 21 times with

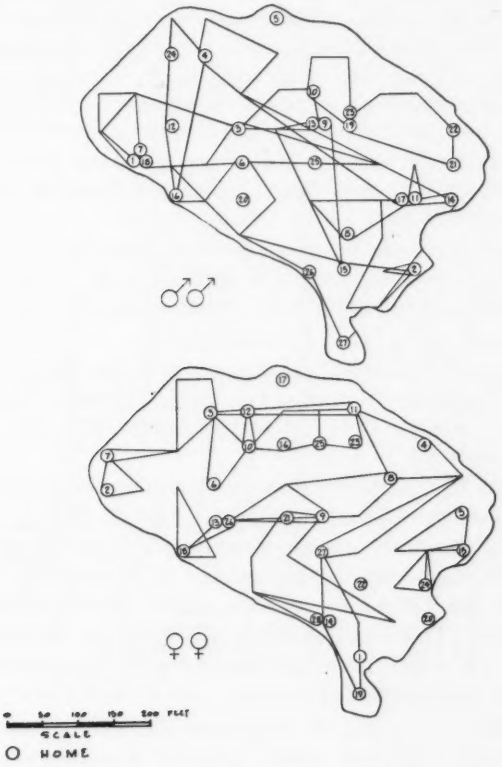



FIG. 6. Homes and ranges of male and female mice in dense population on the experimental island.

77% of the animals captured more than 4 times. Frequency of capture of mice at one trapping station ranged from 2 to 13 times with 73% of the animals captured more than twice. The line arising from a circle joins the outermost points of capture of a mouse and provides an estimate of its range.

Note in Fig. 6 that while there was overlap of the homes and ranges of mice of both sexes, both homes and ranges were fairly uniform in distribution over the island. This observation is made more striking by the obvious differences in habitat for mice on the island. The even distribution of mice, despite the uneven distribution of habitat, suggests that intraspecific strife for space occurred and as one result resident mice were spread over the island.

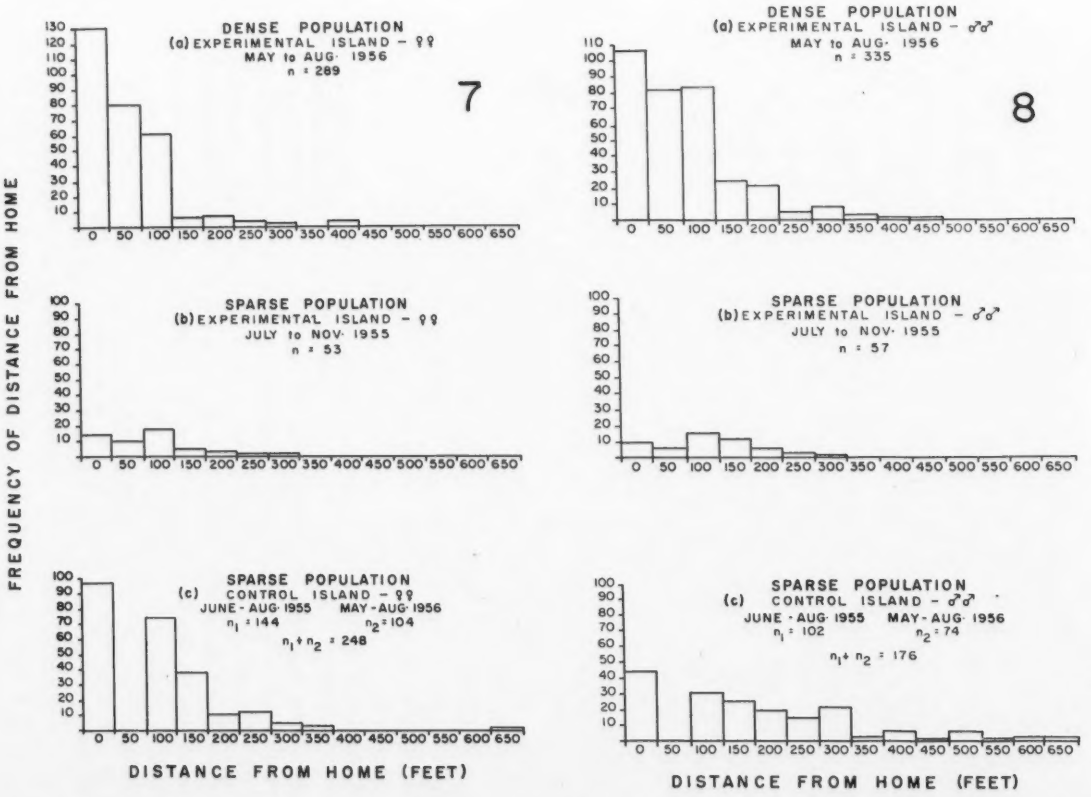
A second argument for intraspecific strife for space in dense population is based on distances moved from home by mice in dense, and relatively sparse populations. Analyses of movements from home were made for males and females in dense population on the experimental island in 1956, and in sparse population on the experimental island in 1955 and control island in 1955 and 1956. In each year, the distances moved from home by individuals of each sex were pooled to give movements of males or females from home. The distances that males or females moved from home were then plotted against frequency to give a histogram of movements for each of the populations noted above. The histograms of movement were tested for difference by chi-square, $R \times C$ test at the 0.05 level of probability. There was no statistically significant difference between the histograms of movement of male or female mice on the control island in 1955 and 1956. Hence, the data for 1955 and 1956 from the control island were pooled by sex.

The results of the analysis of movement from home are presented in Figs. 7 and 8. The absence of movements of 50 ft from the histograms of movement of mice on the control island is the result of the 100-ft spacing between traps of the area.

Note in the figures that males tended to move greater distances from home than did females in dense or sparse population. This is in keeping with the belief that the range of the male *P. leucopus* is larger than that of the female (Burt (3), Townsend (37)).

The movements of animals in sparse population on the experimental island in 1955 and on the control island are shown in Figs. 7*b*, 8*b*, and 7*c*, 8*c*. There is no statistically significant difference between the movements of males or females in sparse population on the islands. In the test for difference between islands in the distances moved by males, movements beyond 350 ft were not included. This manipulation is to correct for the small size of the experimental island which does not permit the long movements of males recorded on the control island.

Note now Figs. 7*a* and 8*a*; the frequency distributions of the distances moved from home by male and female mice in dense population. The figures are more negatively skewed. The differences between histograms of movements of male and female mice in dense population and of male and female




FIGS. 7 and 8. Frequency of movements from home of females (Fig. 7) and males (Fig. 8): (*a*) in dense population on the experimental island, (*b*) in sparse population on the experimental island, (*c*) in sparse population on the control island.

mice in sparse population are statistically significant. Hence, in dense population mice tended to move shorter distances from home than in sparse population.

In the test for difference between movements of females, a significantly large chi-square value was obtained from the zero class of distance moved from home. This was not the case in the males, which suggests that the movements of females in dense population were more restricted than those of males.

There is the possibility that the different spacing of traps on the islands affected the above results. Hayne (18) has demonstrated with *Microtus* that the size of home range is a positive function of the distance between traps. However, the mean distance that females moved from their centers of activity showed little or no change with an increased distance between traps. This was not true of the males where mean distance increased with increased distance between traps. In the present study, the similarity of movements of mice in sparse population on both islands, and the difference in movements of mice in sparse and dense populations on the same island, indicate that trap spacing did not affect the above results. Moreover, in the case of the females, the significant chi-square value from the zero class of movement from home strongly suggests that the effect of trap spacing was unimportant as a cause of difference in movement.

From the analysis of the spatial relationships of mice, animals in dense population were distributed rather uniformly over the experimental island, and the distances that mice moved from home decreased with increased density of population. These results suggest the development of intraspecific strife for space in dense population. It is known that extreme overcrowding in populations of small mammals results in increased death rates (Calhoun (5), Southwick (30), Louch (24), and others) and decreased birth rates (Clarke (10), Southwick (29), and others) as manifestations of intraspecific strife. Burt (3) calls attention to territorial behavior in the white-footed mouse and suggests that competition for space causes dispersal. Hence, intraspecific strife can regulate population, and intraspecific strife for space appears to be a factor that was operating in dense population of mice on the experimental island. We conclude that intraspecific strife for space may become a limiting factor to population of white-footed mice beyond a density determined by the supply of food.

## Discussion

The general conclusions from this work are: (1) the supply of food is an important factor controlling the abundance of white-footed mice in nature, (2) food supply regulates population of mice by affecting death rate (death and dispersal), and possibly birth rate by advancing the time of first breeding in females. The most important effect of food supply is on the survival of young from birth to approximately 1 month of age, and (3) intraspecific strife for space may regulate population of mice beyond a density determined by the supply of food.

How food supply affects the survival of young is unknown, although numerous explanations come to mind and form a basis for further work.

Other workers have concluded that food supply is an important factor determining the abundance of small mammals (Brown (2), Pearson (27), Thompson (36), and others). Jameson (22) believes food supply to be an important factor in population fluctuations of *Peromyscus boylei* and *Peromyscus maniculatus* in the Sierra Nevada, California. His data indicate that the supply of food affects breeding activity, and in turn the abundance of mice. Our data suggest that the most important effect of food supply is on the survival of young. A recent study of *Microtus* populations by Hoffman (19) indicates that the survival of young is a major factor in the control of population. However, the relationship of food supply to survival of young was not reported.

While food supply is a factor limiting the abundance of white-footed mice, it is not the only factor affecting population. Time of breeding is adjusted to the seasons (Whitaker (38)), and this adjustment is apparently independent of the supply of food. Hence, reproductive activity, and in turn population, are affected by factors governing time of breeding.

Then there is evidence that weather affects the abundance of mice by modifying the effect of food supply on the survival of young. For this discussion consider the productivity of females on the experimental island by May of 1956, and on the control island by May of 1955. Thirty-one adult females on the experimental island produced 64 young, of an age taken in traps, for 2.1 young per female. From autopsy data it appears that all the females were breeding and average litter size was 5.2 young. The point here is that despite an excess of food, the production of young was less than half the production that might be expected.

On the other hand, if data from the control island for June of 1955 are extrapolated to read the productivity of females in May then a ratio of 3.0 young per adult female is obtained. This number of young to adult females is significantly greater than on the experimental island with food in excess (chi-square, $2\times2$ table, $P > 0.05$), and certainly greater than the ratio of young to adult females on the same island by May of 1956. Recall that there was no difference in death rate of mice taken in live traps on the two islands in 1955 and 1956. Apparently, some factor or factors were acting to modify the effect of food supply on the survival of young. It is argued that had food in excess been on the experimental island in May of 1955, the survival of young would have been greater than the survival of young on the control island in the same year, and on the experimental island in 1956.

As noted above, the spring of 1956 as compared with 1955 was late with cold temperatures, high winds, and snow. It seems reasonable to believe that this weather adversely affected the survival of young on both the experimental and control islands. From this analysis, weather may modify the effect of food supply on the survival of young, and in turn, modify the growth of population.

Finally, the steady loss of mice of 1 month of age and older from the populations tended to decrease population. This loss appears to be independent of food above some low level of supply. The loss cannot be explained, other than to speculate that it was the result of predation and dispersal. Since mice were repeatedly trapped in a particular area, it would appear that predation is the most important cause of loss of older mice. However, there is evidence that intraspecific strife for space occurs in dense population. Under this circumstance, dispersal caused by competition for space, and predation may be important factors limiting the density of white-footed mice.

## Acknowledgments

It is indeed a pleasure to formally thank the people and institutions that have assisted in this study. Mr. R. D. Lisk assisted in all phases of the work in the field and laboratory. Mr. R. Hurley worked on the project for one year. From time to time, Messrs. S. Teeple, J. Hart, and J. Lake participated in the gathering of data.

Dr. S. Nash of the Department of Mathematics, University of British Columbia, provided some statistical advice, and Dr. R. S. Freeman of the Department of Parasitology, Ontario Research Foundation, identified most of the parasites mentioned in the text. Mr. G. Holland, Science Service, Ottawa, identified *O. leucopus*.

Dr. I. McT. Cowan and Mr. R. Y. Edwards read the manuscript and offered constructive criticisms.

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# ON AN UNUSUAL FISH TAPEWORM FROM MAN[1]

GLORIA A. WEBSTER AND T. W. M. CAMERON

## Abstract

An abnormal specimen of *Dibothriocephalus* from a boy in Saskatchewan has segments much longer than broad, as well as a few abnormal triangular ones. It is suggested that this may be the result of anthelmintic action or the physiological condition of the host.

A portion of a pseudophyllidean cestode collected from a patient in the Prince Albert Sanatorium, Saskatchewan, was forwarded to us.

Stool examinations of the patient, a 7-year-old Indian boy, had repeatedly shown ova of *Dibothriocephalus*. On March 15 and May 28, 1956, he received treatment and passed two fish tapeworms, measuring 13 and 15 ft, respectively; in both worms the scolex was lacking. As ova were still appearing in the stool in August, a third treatment was given on September 19. The portion of the tapeworm recovered the following day was so different from those portions collected on the two previous occasions that it was identified tentatively at the hospital as *Taenia* sp., on the basis of the size and shape of the gravid proglottids, which differed significantly from those of *Dibothriocephalus latus* typically found in man.

The strobila measures 6 ft and consists entirely of mature proglottids, each segment being nearly twice as long as broad (7.9 to 5.4 mm average) (Figs. 1–2). Irregular segmentation has resulted in the interpolation of smaller triangular segments (Fig. 3), some of which contain genitalia (Fig. 4), but there is no duplication of genitalia in any one segment. The genital pore lies 1.7 mm (average) from the anterior margin and is surrounded by a translucent zone. The irregular testes, disposed in a single layer, occupy the lateral fields and are not confluent in either the anterior or posterior sectors of the segment. The ovary is a reticulate U-shaped structure the two anterior projections of which are joined by a massive connection of ovarian material rather than by a narrow isthmus. There are five to eight lateral uterine coils which never project beyond the limit of the genital pore. In segments in which it could be observed the Mehlis gland is a compact sphere which appears to be embedded in the ovary but is actually dorsal to it. Like the testes, the vitelline glands are distributed in the lateral fields and are not confluent anterior to the genital pore or in the posterior portion of the proglottid. The eggs measure 56 by 40 $\mu$ (average).

Elongate segments are not unusual in the genus *Dibothriocephalus* but, except for a few species in which the segments are characteristically longer than broad, this type of proglottid is usually associated with the age of the strobila. In studying the development of *D. latus* in dogs Wardle and McColl

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(3) noted that the early or 'primary' strobila consisted of proglottids with a length 1.5 to 2.0 times the width. These authors found that except in the case of superinfected animals the 'secondary' strobila, with segments broader than long, developed by the third week after the initial infection. Since this observation other investigators have recognized the existence of two strobilar forms in other species. In reviewing the history of this patient we consider it improbable that the elongate segments in this strobila can be considered as forming part of a primary strobila.

It is well known that there are extreme morphological variations within a single species of *Dibothriocephalus*. Stunkard (2) stated: "The extent of variation which may occur normally or may be induced by development in unusual hosts must be known before specific limits of *D. latum* can be determined." In comparing tapeworms from artificially infected sea gulls, bears, foxes, dogs, and man, Babero (1) found strong evidence of the involvement of only one species, despite wide variations in gross appearance, stating: "Possibly the host species involved, its physiological condition, and the age of the worm may be primary determining factors in the morphological variations of the adult form." Wardle and McColl (3) suggest that: "Under conditions of light infestation and ample nutrition the proglottids are linear, because the nutritional demands of the reproductive system limit the surplus of material available for tissue growth." Consequently, a retardation of genital development releases material for tissue growth, and quadrate or elongate segments may interrupt the sequence of the linear segments of the secondary strobila.

In the present case we consider it reasonable to assume that the portion of tapeworm recovered after the third treatment was a product of the original infection as the patient had been confined to hospital during the entire period with little or no opportunity for reinfection. We would suggest that the physiological condition of the host, and/or the treatments he received, might in some way have affected the growth of the worm resulting in the production of elongate segments. Regardless of the cause of this anomaly, it is worth noting that the proglottids may take this form in man. There have been occasional reports of *Taenia* infections occurring in areas of Canada where neither beef nor pork is available. On the basis of our material, it would appear that these cases may have been incorrectly diagnosed.

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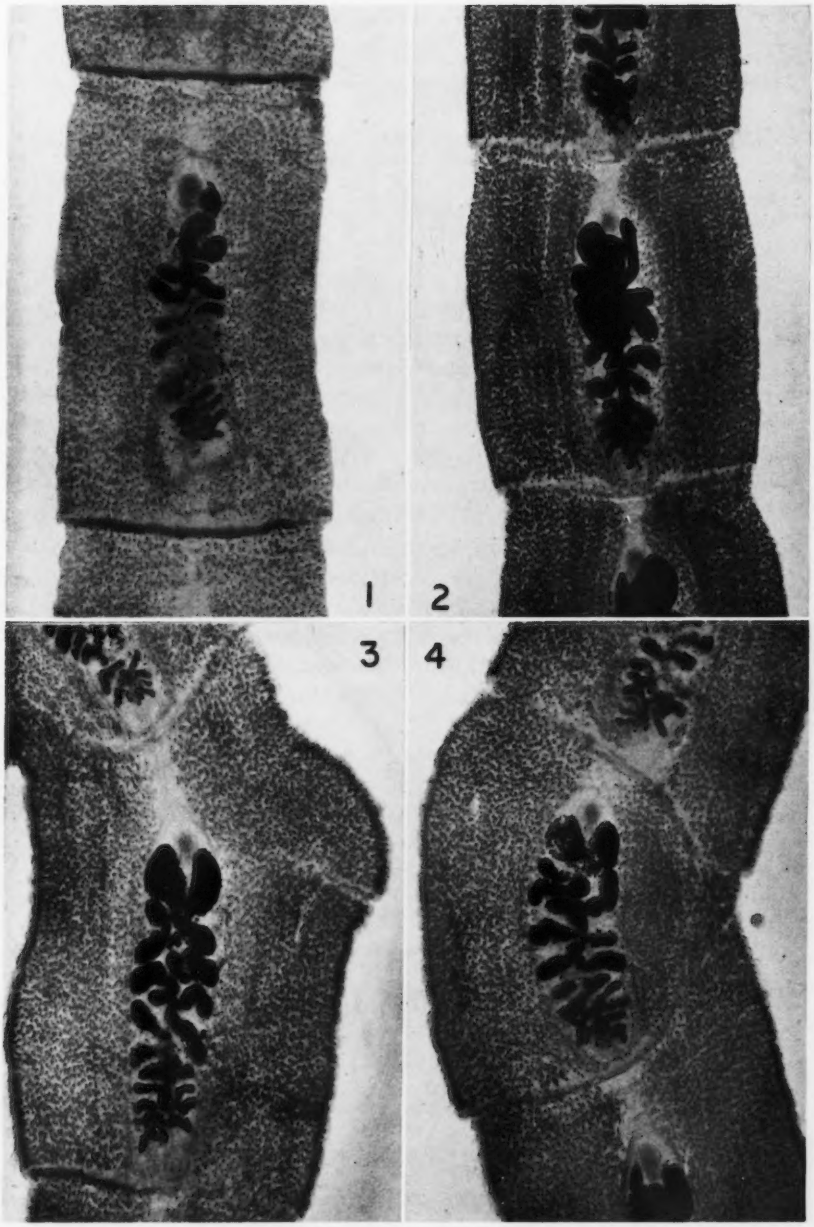


FIG. 1–4. Unusual *Dibothriocephalus* from man. Fig. 1. Elongated segment. Fig. 2. Shortest segment in strobila. Fig. 3. Small triangular addition to edge of elongated segment. Fig. 4. Triangular segment.

Webster and Cameron—Can. J. Zool.

# NOTES

## ORCHIPEDUM TRACHEICOLA REPORTED FROM A WHISTLING SWAN, CYGNUS COLUMBIANUS

GLORIA A. WEBSTER

Parasites collected from the trachea of a juvenile whistling swan (*Cygnus columbianus*) have been identified as *Orchipedum tracheicola* Braun, 1901. The bird was observed flying alone during the fall around Cooking Lake near Edmonton, Alberta, and was thought to be ill; ultimately it was found frozen in the ice. At post-mortem examination numerous trematodes were found in the trachea; in addition, acanthocephala were collected from the middle and lower intestine. Dr. C. H. Bigland, of the Veterinary Laboratory, Alberta Department of Agriculture, Edmonton, forwarded the specimens to the Institute of Parasitology for identification.

*Orchipedum tracheicola* was first recorded from the trachea of a velvet scoter (*Melanitta fusca*) in Europe by Braun (1); subsequently it was reported from eastern North America by Cheatum (2), who found these parasites in the respiratory tract of a white-winged scoter (*Melanitta deglandi*). Cowan (3) collected a single specimen of *O. tracheicola* from a trumpeter swan (*Cygnus buccinator*), captured in British Columbia; this was the first record of its occurrence in this host.

Other species of *Orchipedum* have been reported in Europe from cranes, herons, spoonbills, pelicans, and coots. The genus is apparently not common in North America, and this appears to be the first record of *O. tracheicola* from a whistling swan.

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# THE CHEMICAL COMPOSITION OF THE GASTRIC SHIELD OF THE OYSTER CRASSOSTREA VIRGINICA (GMELIN)

BARBARA L. SHAW AND HELEN I. BATTLE

The gastric shield of *Crassostrea virginica* is a bilobed laminated structure composed of an opalescent translucent material superficially resembling the matrix of hyaline cartilage, although exhibiting an acidophilic rather than a basophilic reaction to stains. Shaw and Battle (10) were in agreement with Nelson (8) in describing it as "chondroid-like". Berkeley (1), on the contrary, demonstrated the chitinous nature of the gastric shield of the lamellibranch *Schizothoerus nutalli* and concluded that it is lacking in any chondrin-like constituent.

Structurally the molluscan gastric shield in certain respects may be analogous to the peritrophic membrane of insects, which Wigglesworth (11) has shown, in some instances, to be composed of concentric lamellae secreted by the underlying epithelial cells of the mid-gut. It has been demonstrated that the gastric shields of a number of molluscs are resultant from a fusion of droplets of secretion from underlying epithelial cells, viz.: *Mytilus* (List (7)), *Anodonta* (Gutheil (4)), *Ostrea chilensis* (Dahmen, (3)), *Ostrea angulata* (Leenhardt, (6)). Yonge (12), however, has supported the viewpoint that the shield in *Ostrea edulis* is not a secretion but is formed by fusion of cilia in response to the irritation caused by the head of the style. In *C. virginica*, cytoplasmic strands are evident between the gastric shield and the underlying gastric epithelium. Hence, if the shield represents a product of epithelial cells, rather than being comparable to an amorphous connective tissue-ground substance, as inferred in the description "chondroid-like", the statement of Shaw and Battle (10) merits re-examination. Accordingly shields of this species have been subjected to various chemical tests for chitin.

Richards (9) has shown that pure chitin, like many other substances, is colored brown by iodine but chitosan in an acidified medium takes on a violet or a reddish-violet coloration. Since very few organic substances will withstand the alkaline treatment necessary to convert chitin to chitosan, he considers that this iodine–chitosan color test is completely valid when positive. Gastric shields of *C. virginica*[1] were dissected entirely free from adhering gastric epithelial tissues and treated according to the method of Campbell (2): small test tubes containing shields in 5 ml of KOH saturated at room temperature were closed with a Bunsen valve and slowly heated in a glycerine bath to 160° C for 15 minutes. On microscopic examination after they were cooled to room temperature, shields invariably appeared quite intact and of characteristic shape. They were transferred to water and dehydrated by passage through a graded series of alcohols prior to drying.

[1]Supplied by Fisheries Research Board of Canada.

The following characteristic reactions for chitin transformed to chitosan were positive in each instance:

(*i*) Slow solution in 3% acetic acid and appearance of a white precipitate on addition of 1% sulphuric acid.

(*ii*) Brown coloration appeared rapidly in 0.5% iodine in potassium iodide. Following removal of excess iodine solution and replacement by 1% sulphuric acid, the edges of the shields became reddish violet and the central thicker area almost blackish. On replacement of the dilute acid by 75% sulphuric acid, the color gradually faded to yellow at the edges and the whole shield ultimately became colorless.

In addition, shields stained a dark brownish black in pyrogallic acid after they were boiled in 60% potassium hydroxide, indicating a positive reaction for chitin according to the method of Kennedy (5).

On the bases of the above tests, there would seem to be positive evidence that the gastric shield of *C. virginica*, like that of *Schizothoerus nutalli* (Berkeley (1)), is chitinous rather than chondroid in nature.

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